JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 1577−1583 **1577**

Enantiomeric Resolution of Chiral Pesticides by High-Performance Liquid Chromatography

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Successful enantiomeric separation of 10 chiral pesticides by high-performance liquid chromatography (HPLC) using cellulose-tris(3,5-dimethylphenylcarbamate) (CDMPC) chiral stationary phase (CSP) was performed. The mobile phase was *n*-hexane modified by ethanol, propanol, 2-propanol (IPA), butanol, or isobutanol. The effects of mobile phase composition and column temperature on the separation were investigated. Baseline separation was obtained with ethofumesate, fluroxypyr-meptyl, malathion, benalaxyl, diclofop-methyl, methamidophos, vinclozolin, and lactofen, whereas near baseline separation was obtained with profenofos and acetochlor. Butanol was the best modifier for benalaxyl; isobutanol was the best modifier for lactofen, malathion, diclofop-methyl, and ethofumesate; and IPA was the best modifier for the other five. Better separations were not always at low temperature. The elution orders of the eluting enantiomers were determined by a circular dichroism (CD) detector. The quantitative analysis methods for the enantiomers of ethofumesate, benalaxyl, and diclofop-methyl were established. Validation parameters include linearity, precision, and limit of detection (LOD). The enantiomeric residual analysis procedures in soil and water samples were also developed using acetone extraction and C_{18} solid phase extraction. The methods were reliable for residual analysis of the enantiomers.

KEYWORDS: Enantiomeric resolution; HPLC; chiral pesticides

INTRODUCTION

Chiral pesticides have attracted great attention in recent years (*1*), and the number of optical purity pesticides reaching the market place has been increasing (*2*, *3*). Enantiomers have identical physical and chemical properties, but their behaviors in biological systems could be completely different (*4*−*6*). There is an urgent need to develop analytical methods to determine the optical purity, stereoselective bioactivity, and environmental behavior of chiral pesticides.

According to the previous references, the two enantiomers of ethofumesate have been separated by capillary electrophoresis using sulfobutyl ether β-cyclodextrin as a chiral selector (*7*). Malathion was resolved by mixed-mode electrokinetic capillary chromatography (*8*) and chiralcel OJ chiral stationary phase (CSP) (*9*). Profenofos was separated by an AD column (*10*). Diclofop-methyl was separated on cellulose tris(4-methylbenzoate) CSP (*11*) and permethylated β-cyclodextrin high-performance liquid chromatography (HPLC) and gas chromatography (GC) columns (*9*).

Ten chiral pesticides were separated in this study including three insecticides (malathion, profenofos, and methamidophos), two fungicides (benalaxyl and vinclozolin), and five herbicides (diclofop-methyl, lactofen, ethofumesate, fluroxypyr-meptyl, and acetochlor). The chemical structures of these compounds are shown in **Figure 1**. The chirality of acetochlor was due to the asymmetrical axis, and the other samples were from the asymmetrically substituted carbon or phosphorus atom. All of the pesticides consisted of two enantiomers. The chiral separations were performed by a robust CSP cellulose-tris(3,5-dimethylphenylcarbamate) (CDMPC) (*12*−*14*) in an HPLC system. A *n*-hexane mobile phase with polar organic alcohols including ethanol, propanol, 2-propanol (IPA), butanol, and isobutanol was used. The effect of temperature on the resolution was investigated. Circular dichroism (CD) information was used to assign the elution orders of the eluting enantiomers. The CSP gave good separations for most of the chiral pesticides with excellent repeatability and was capable of preparation in small scale.

The quantitative analysis of the single enantiomers of three chiral pesticides ethofumesate, benalaxyl, and diclofop-methyl and residual analysis in soil and water samples were also performed in the study.

The purpose of the work is to set up methods for quantitative and residual analysis of the chiral pesticide enantiomers, allowing optical purity determination and further research on the steroselective behaviors in the environment.

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10.1021/jf052631o CCC: $33.50
Published on Web 02/11/2006

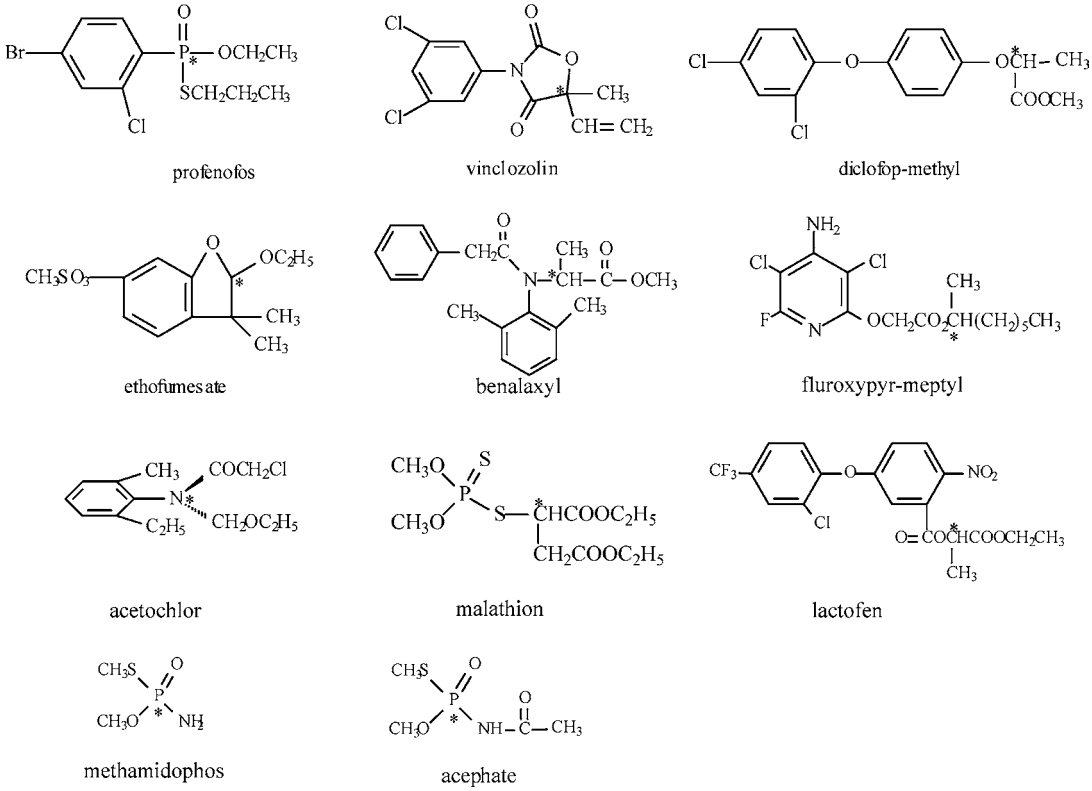

Figure 1. Chemical structures of the chiral pesticides; an asterisk denotes a chiral center.

MATERIALS AND METHODS

Chemicals. Microcrystalline cellulose and 3,5-dimethylphenylisocyanate (99%) were purchased from Sigma-Aldrich Inc. (United States). 3-Aminopropyltriethoxysilane (99%) was from Acros Organics (Belgium). Macrospherical silica was prepared in this laboratory with the following properties: particle size, 5−7 μm; average pore diameter, 6.7 nm; and specific surface area, 110 $m^2\ g^{-1}$. Ethofumesate was provided by Jiangsu Good Harvest-Weien Agrochemical Ltd. (China), fluroxypyr-meptyl was from Trustchem Co. Ltd. (China), and malathion was from Guangxi Yulin Jintudi Pesticide Co. Ltd. (China). Other pesticide samples were supplied by the Institute for Control of Agrochemicals Ministry of Agriculture (Beijing, China). All eluents were of analytical grade (Beijing Yili Fine Chemicals Co., Ltd.), distilled, and filtered (0.45 μm) before use.

Apparatus. *System 1.* Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA) equipped with G1311A pump, G1322A degasser, G1328A injector, G1316A COLCOM, a 20 μL sample loop, and G1315B DAD. The signal was acquired and processed by an HP1100 workstation.

System 2. JASCO 2000 HPLC (Jasco Co., Tokyo, Japan), equipped with pu-2089 plus pump, CD-2095 plus CD detector, a 20 μL sample loop, and Chrompass workstation was used. This system was used to determine the elution orders of the enantiomers. Solid phase extraction (SPE) vacuum manifold (Supelco, United States) C_{18} cartridges (500 mg, Agilent) were also used.

Chromatographic Conditions. The column was 250 mm × 4.6 mm (i.d.). The mobile phase was *n*-hexane with the addition of ethanol, *n*-propanol, IPA, *n*-butanol, or isobutanol as a modifier. The flow rate was 1.0 mL min^{-1}, and the injection volume was 20 μL. The monitoring wavelength was listed in **Table 1**. The influence of column temperature was studied using *n*-hexane−IPA mobile phase. The capacity factor [$k' = (t - t_0)/t_0$], separation factor ($\alpha = k_1'/k_2'$), and resolution factor ($Rs = \{[2(t_2 - t_1)]/(w_1 + w_2)\}$) were calculated.

Preparation of CSP. The CSP was synthesized according to refs *15* and *16*. Macrocrystalline cellulose reacted with 3,5-dimethylphenylisocyanate in pyridine at 110 °C for 24 h to synthesize CDMPC. After it was cooled to room temperature, the product was precipitated by methanol, filtered, and dried at 60 °C for 12 h. Aminopropylsilica (APS) was synthesized by treating spherical silica with 3-aminopropyltriethoxysilane in toluene. The CSP was prepared by coating CDMPC to APS. The slurry of the CSP in *n*-hexane−IPA (90:10 v/v) solution was packed into a stainless steel column under 4.0×10^7 Pa.

Quantitative Analysis of the Enantiomers. The stock standard solutions were prepared by dissolving the racemic pesticide samples in IPA and then were diluted to a series of concentrations (ethofumesate: 241.92, 48.38, 4.84, 0.48, and 0.24 mg L^{-1}; benalaxyl: 259.2, 129.6, 64.8, 5.2, and 0.5 mg L^{-1}; diclofop-methyl: 256.1, 51.2, 5.12, 0.51, and 0.26 mg L^{-1}). Because two enantiomers in racemic samples were in the ratio of 1:1, the concentration of each enantiomer was thus known. The standard solutions were injected in triplicate (20 μL) to evaluate the linearity, relative standard deviation (RSD), and limit of detection (LOD). The linearity was obtained based on the plot of concentration vs peak area.

Extract from Soil. Known blank soil samples (25 g, dried at room temperature) were fortified by the addition of the pesticide standard solutions to give three concentration levels of the enantiomers. After the addition of 10 mL of purified water (purified by MilliPore water purification system) and shaking for 1 min, the soil sample was allowed to equilibrate for 1 h prior to extraction. The enantiomers were extracted with 40 mL of acetone, with addition of 10 mg of activated carbon. After it was shaken for 0.5 h, the mixture was filtered and the residual soil was extracted by another 25 mL of acetone. The filtered solvent was combined into a round-bottomed flask and evaporated to remove most of the acetone on the rotary evaporator (40°C, reduced pressure). The residual solution was transferred to a separating funnel, 5 mL of saturated sodium chloride solution was added, and this mixture was extracted by 30, 15, and 10 mL of dichloromethane. The solvent was evaporated at 35 °C on the rotary evaporator to near dryness, and the last trace was removed by a gentle stream of nitrogen. The extract was dissolved in IPA (1 mL). Triplicate analyses were performed for each fortification level.

Extract from Water. A SPE method was used to extract the enantiomers in water by C_{18} cartridges (500 mg, 6 mL). The water samples were prepared by adding the pesticide standard solutions to 100 mL of purified water and were allowed to equilibrate for 1 h. The extraction columns were conditioned by 10 mL of methanol and then 15 mL of purified water. The water sample was loaded and passed through the column at a flow rate of about 2 mL min^{-1}. The pesticides

Table 1. Chiral Separations and the Effect of Modifiers at Room Temperature

compounds	modifier	content (%)	k_1'	α	Rs	compounds	modifier	content (%)	k_1'	α	Rs
profenofos	ethanol	0.5	7.62	1.00	0	malathion	ethanol	1.0	1.78	1.13	0.67
(210 nm)	propanol	0.5	7.29	1.07	0.94	(210 nm)	propanol	1.0	1.93	1.25	1.29
	IPA	0.5	8.88	1.10	1.35		IPA	1.0	2.08	1.30	1.44
	butanol	1.0	2.64	1.05	0.61		butanol	1.0	1.72	1.12	0.73
	isobutanol	1.0	2.92	1.07	0.79		isobutanol	1.0	2.11	1.34	1.50
vinclozolin	ethanol	1.0	2.17	1.12	1.10	acetochlor	ethanol	1.0	2.68	1.11	0.87
(210 nm)	propanol	1.0	2.37	1.12	1.12	(230 nm)	propanol	1.0	2.82	1.11	0.89
	IPA	1.0	2.83	1.14	1.46		IPA	1.0	3.20	1.14	1.16
	butanol	1.0	2.57	1.13	1.16		isobutanol	1.0	2.84	1.12	0.91
	isobutanol	1.0	2.54	1.12	0.96	fluroxypyr-meptyl	ethanol	1.0	2.49	1.18	1.06
diclofop-methyl	ethanol	2.0	1.34	2.37	3.55	(230 nm)	propanol	1.0	2.60	1.17	1.01
(230 nm)	propanol	2.0	1.44	2.59	5.62		IPA	1.0	2.99	1.26	1.40
	IPA	2.0	1.45	3.14	5.32		butanol	1.0	2.60	1.10	0.60
	butanol	2.0	1.43	1.84	2.88		isobutanol	1.0	2.68	1.10	0.69
	isobutanol	2.0	1.64	3.14	6.15	lactofen	ethanol	1.0	2.80	1.16	0.6
benalaxyl	ethanol	2.0	5.24	1.45	5.00	(254 nm)	propanol	1.0	3.60	1.43	1.73
(230 nm)	propanol	2.0	8.89	1.36	5.02		IPA	1.0	3.79	1.50	1.84
	IPA	2.0	10.82	1.33	4.39		butanol	1.0	3.53	1.27	1.48
	butanol	2.0	8.05	1.73	7.84		isobutanol	1.0	4.63	1.54	1.87
	isobutanol	2.0	9.56	1.49	5.64	methamidophos	ethanol	10.0	2.75	1.23	1.03
ethofumesate	ethanol	5.0	4.07	1.45	5.13	(230 nm)	propanol	10.0	3.10	1.23	1.30
(230 nm)	propanol	5.0	4.36	1.63	5.61		IPA	10.0	4.42	1.27	1.54
	IPA	5.0	5.36	1.78	5.64		butanol	10.0	3.50	1.21	1.32
	butanol	5.0	4.34	1.56	5.42		isobutanol	10.0	4.23	1.23	1.50
	isobutanol	5.0	5.01	1.73	7.05						

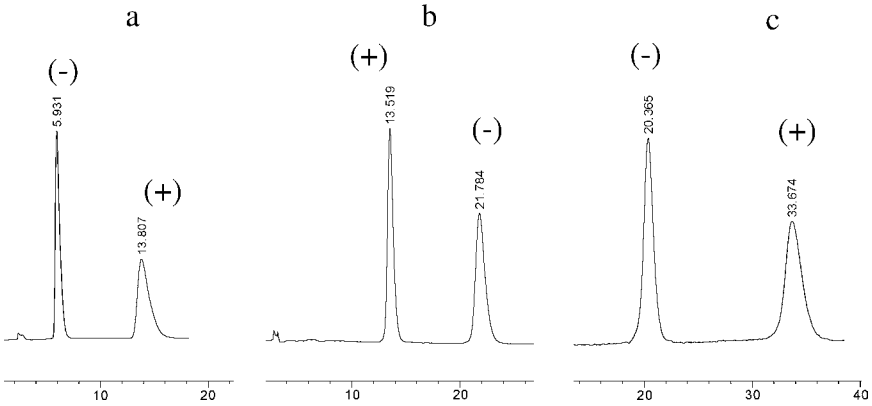

Figure 2. Chromatograms for chiral resolutions at room temperature. (**a**) Diclofop-methyl, 2% isobutanol, 230 nm, and 1.0 mL min^{-1}; (**b**) ethofumesate, 5% isobutanol, 230 nm, and 1.0 mL min^{-1}; and (**c**) benalaxyl, 2% butanol, 230 nm, and 1.0 mL min^{-1}.

were eluted with 5 mL of methanol, and the eluate was evaporated by a nitrogen stream. The extract was dissolved in IPA (1 mL). Triplicate analyses were performed for each fortification level.

RESULTS AND DISCUSSION

Chiral Resolution. The direct resolutions of 10 chiral pesticides were performed. Optimization of the chromatographic condition was done including investigating the effect of the type and concentration of modifier and column temperature on the resolutions. **Table 1** listed the effect of modifiers and their content on the separations at room temperature. The resolution factor Rs was used to evaluate the separation in this study, and it was considered a complete separation when the Rs exceeded 1.5.

The best resolution (Rs = 1.35) of profenofos was achieved using 0.5% IPA; however, the use of ethanol provided no separation. The enantiomers would not elute using 0.5% butanol or iso-butanol. Vinclozolin obtained a better resolution (Rs = 1.46) with 1% IPA, and the resolution became poorer when the content of modifier was lower than 1% because of peak broadening. Except for IPA, other modifiers showed poor chiral selectivity for the two enantiomers of acetochlor, and butanol gave no separation. Methamidophos also showed better separation using IPA modifier. Lactofen could obtain baseline separation using 1% propanol, IPA, or isobutanol in *n*-hexane with Rs values of 1.73, 1.84, and 1.87, respectively, and 1% butanol could give near baseline separation. Ethanol was a poor modifier for the separation. Isobutanol was more suitable for the separation of malathion (Rs = 1.50, 1%), and IPA was also effective. The two enantiomers of fluroxypyr-meptyl obtained the best resolution using 1% IPA (Rs = 1.40), while butanol and isobutanol were poorer modifiers. The two enantiomers could be completely separated using 10% IPA or isobutanol.

Diclofop-methyl, benalaxyl, and ethofumesate showed excellent enatniomeric selectivity on the CSP. Complete resolutions were obtained by using the five alcohol modifiers even at higher concentrations (20%). Isobutanol was the best modifier for diclofop-methyl (Rs = 6.15, 2%) and ethofumesate (Rs = 7.05, 5%), butanol was the best modifier for benalaxyl (Rs = 7.84, 2%). **Figure 2** shows the chiral resolutions. As compared with other modifiers, ethanol was relatively not effective for diclofop-methyl and ethofumesate, and IPA was not effective for benalaxyl.

Table 2. Effect of Temperature on the Separation

compound	hexane/IPA	*T* (°C)	k_1'	α	Rs
profenofos	99/1	5	3.09	1.08	1.07
		10	2.99	1.08	1.00
		15	2.94	1.08	0.99
		20	2.86	1.07	1.07
		25	2.87	1.07	1.08
		30	2.70	1.07	1.05
		40	2.58	1.07	1.02
	99.5/0.5	5	9.84	1.11	1.41
malathion	99/1	5	2.39	1.34	1.50
		10	2.13	1.31	1.43
		15	2.16	1.30	1.45
		20	2.08	1.30	1.44
		25	1.98	1.28	1.41
		30	1.89	1.28	1.40
		40	1.73	1.26	1.40
diclofo*p*-methyl	80/20	5	0.82	2.96	3.22
		10	0.80	2.89	3.19
		15	0.74	2.73	3.48
		20	0.71	2.57	3.07
		25	0.68	2.54	3.20
		30	0.63	2.60	3.19
		40	0.52	2.24	2.39
ethofumesate	85/15	5	3.23	1.71	5.68
		10	3.07	1.68	5.58
		15	2.86	1.64	5.44
		20	2.67	1.60	5.26
		25	2.51	1.56	5.01
		30	2.34	1.53	4.83
		40	2.10	1.47	4.37
acetochlor	99/1	5	3.64	1.16	1.15
		10	3.54	1.15	1.10
		15	3.41	1.15	1.09
		20	3.20	1.14	1.16
		25	2.97	1.14	1.14
		30	2.79	1.13	1.14
		40	2.58	1.13	1.17
fluroxypyr-meptyl	99/1	5	3.63	1.33	1.76
		10	3.44	1.31	1.72
		15	3.21	1.28	1.59
		20	2.99	1.26	1.49
		25	2.74	1.23	1.36
		30	2.55	1.20	1.24
		40	2.32	1.17	1.12
methamidophos	90/10	5	5.21	1.34	1.74
		10	4.95	1.32	1.77
		15	4.62	1.32	1.80
		20	4.43	1.31	1.66
		25	4.22	1.30	1.58
		30	4.00	1.30	1.55
		40	3.71	1.29	1.59
lactofen	90/10	5	2.38	1.48	1.69
		10	2.19	1.46	1.58
		15	2.02	1.46	1.53
		20	-	-	-
		25	1.73	1.43	1.36
		30	1.55	1.42	1.28
		40	1.44	1.39	1.18
vinclozolin	99/1	5	2.82	1.29	1.38
		10	2.62	1.40	1.50
		15	-	-	-
		20	2.53	1.32	1.41
		25	2.41	1.45	1.55
		30	2.32	1.45	1.55
		40	2.22	1.31	1.40
benalaxyl	85/15	5	3.16	1.32	2.42
		10	2.97	1.30	2.36
		15	2.72	1.28	2.30
		20	2.39	1.25	2.15
		25	2.36	1.24	2.12
		30	2.12	1.23	1.97
		40	1.92	1.21	1.91

Effect of Modifiers. The retention of the eluting enantiomers using different modifiers in mobile phase is in the order of ethanol < propanol < butanol < isobutanol < IPA, which is inconsistent with the polarity or viscosity. Organic alcohol modifiers compete with the solutes for the interactions with the CSP (*17*, *18*), and a low concentration of modifiers results in the strong retention and higher resolution. The results suggested that the polarities and viscosities are not the only factors influencing the chiral separation. The interactions between the alcohol molecules and the CSP may also play an important role for the chiral recognition.

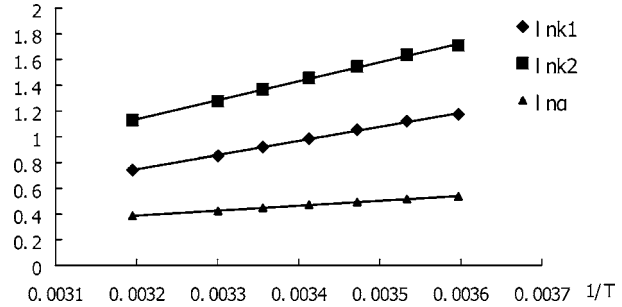

Figure 3. Van't Hoff plot of ethofumesate, *n*-hexane/IPA 85:15.

Table 3. Enantioselectivity and the Elution Order of the Chiral Pesticides on the CSP

compound	hexane/IPA	WL (nm)	elution order peak 1/peak 2
profenofos	99/1	225	±
diclofop-methyl	90/10	254	∓
ethofumesate	90/10	280	±
acetochlor	98/2	225	∓
fluroxypyr-meptyl	90/10	254	∓
lactofen	98/2	270	∓
vinclozolin	99/1	225	±
benalaxyl	90/10	225	∓
malathion	98/2	220	±
methamidophos	95/5	220	±

Chiral Recognition. CDMPC contains a β-polymeric chain of derivatized D-(+)-glucose residues in a β-1,4-linkage. The chains lie side by side and exist in a helical structure in which there are chiral cavities that play an important role in the recognition of particular enantiomers. 3,5-Dimethylphenyl carbamate groups are located outside the cavities (*17*). It is commonly considered that hydrogen bonding, $\pi-\pi$, and dipole−dipole were the main interactions for the stereoselctivity (*7*, *9*).

The structures of the analytes that obtained resolutions all have an electronegative atom (nitrogen, oxygen, or sulfur), C═O group, or phenyl ring directly attaching to the chiral center, which may interact with the CSP through hydrogen bonding, dipole−dipole, or $\pi-\pi$ interactions. These atoms or groups may play important roles in the enantiomeric recognition. For methamidophos and malathion, which contain no aromatic rings, hydrogen bonding and dipole−dipole interactions may cause the chiral resolutions. Methamidophos obtained good separation, while acephate (a hydrogen atom on the amino group of methamidophos, which was substituted by acethyl, shown in **Figure 1**) showed no enantioselectivity. The amino group attaching to chiral center may interact with the CSP by hydrogen bonding, and this interaction would be reduced or hindered by the substituted group. The difference of hydrogen bonding between the two enatiomers and the CSP may attribute to chiral resolution of methamidophos.

Effect of Temperature. The effect of temperature on the separations was investigated applying the *n*-hexane−IPA mobile phase. For diclofop-methyl, benalaxyl, ethofumesate, metha-

Table 4. Validation of the Quantitative Analysis Method for the Enantiomers[a]

pesticide	E	concentration range (mg L^{-1})	linear equation	R	RSD (%)	LOD (mg L^{-1})	chromatographic conditions
ethofumesate	E1	241.92–0.24	$y = 24.0x + 20.3$	0.99	<4.9	0.06	*n*-hexane/IPA 90:10, 230 nm, 1.0 mL min^{-1}, 20 μL, room temperature
	E2	241.92–0.24	$y = 23.9x + 19.2$	0.99	<7.5	0.06	
benalaxyl	E1	259.20–0.52	$y = 65.5x + 119.4$	1	<4.0	0.13	*n*-hexane/IPA 85:15, 206 nm, 1.0 mL min^{-1}, 20 μL, room temperature
	E2	259.20–0.52	$y = 61.6x + 240.4$	0.99	<4.0	0.13	
diclofop-methyl	E1	256.10–0.26	$y = 56.1x + 32.4$	1	<4.0	0.05	*n*-hexane/IPA 90:10, 230 nm, 20 μL, 1.0 mL min^{-1}, room temperature
	E2	256.10–0.26	$y = 56.1x + 31.6$	1	<6.3	0.05	

[a] E1 and E2 represent the first and second eluted enantiomers, respectively.

midophos, and lactofen, which showed good enantioselectivity on the CSP, a higher concentration (10−20%) of modifier in the mobile phase was used in order to save time. For acetochlor, malathion, fluroxypyr-meptyl, profenofos, and vinclozolin that obtained near baseline or partial separations at room temperature, a low content (1 or 2%) of modifier was used. The experiment was performed at a range of 5−40 °C. **Table 2** lists the results. Profenofos obtained near complete resolution ($\alpha = 1.11$, Rs = 1.41) at 5 °C in the mobile phase 99.5/0.5 *n*-hexane/IPA. Malathion and fluroxypyr-meptyl had baseline separations at 5 °C with Rs = 1.50 and 1.76, respectively. The capacity factor (k') and separation factor (α) increased with decreasing temperature for all of the chiral pesticides, but the resolution factor (Rs) did not always increase with decreasing temperature. Diclofop-methyl and methamidophos had higher Rs values at 15 °C, vinclozolin at 30 °C, and acetochlor at 40 °C, not at a low temperature of 5 °C.

It is suggested that at least two different effects of temperature can affect chiral separation (*19*). One is a kinetic effect that influences the viscosity and the diffusion coefficient of the solute. Another is the thermodynamic effect that changes the separation factor. The fact that the separation factor usually decreases with increasing temperature may be because the Gibbs free energy change (ΔG) of transfer of the analyte between the stationary phase and the mobile phase decreases at high temperature.

The enthalpic and entropic contributions to enantioselectivity may be described using the following van't Hoff equations (*18*, *20*−*22*):

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

and

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

where ΔH and ΔS are the standard enthalpy and entropy of transfer of the solute from the mobile phase to the stationary phase. If plots of ln k vs $1/T$ (van't Hoff plots) are linear, the slope and intercept are $-\Delta H/R$ and $\Delta S/R + \ln \varphi$ (ΔS^*), respectively. $\Delta_{R,S}\Delta H^\circ$ and $\Delta_{R,S}\Delta S^\circ$ are the differences $\Delta H_2 - \Delta H_1$ and $\Delta S_2 - \Delta S_1$, respectively. For a linear plot of ln α vs $1/T$, the slope and intercept are, respectively, $-\Delta\Delta H/R$ and $\Delta\Delta S/R$. Linear van't Hoff plots for many enantiomeric separations were reported, and nonlinearity was also found (*23*). **Figure 3** shows the linear van't Hoff plots of ethofumesate, and the linear equations were $\ln k_1 = 1092.5/T - 2.75$ ($R = 0.99$), $\ln k_2 = 1475.1/T - 3.6$ ($R = 0.99$), and $\ln \alpha = 382.6/T - 0.8$ ($R = 0.99$). The parameters such as $\Delta_{R,S}\Delta H^\circ$ and $\Delta_{R,S}\Delta S^\circ$ were −3.2 kJ mol^{-1} and −7.0 J mol^{-1} K^{-1}, respectively.

Table 5. Recovery and Precision of the Enantiomers from Soil and Water ($n = 3$)[a]

		E1 (first eluted enantiomer)		E2 (second eluted enantiomer)	
sample	concentration (mg kg^{-1} or mg L^{-1})	recovery (%)	RSD (%)	recovery (%)	RSD (%)
		ethofumesate			
soil	2.5	99.4	3.2	112.4	1.5
	0.5	103.0	3.8	103.9	1.0
	0.1	111.9	4.3	112.7	2.2
water	0.5	108.4	5.9	112.4	6.2
	0.05	106.2	1.8	119.6	3.2
	0.005	118.4	1.8	122.1	7.9
		benalaxyl			
soil	5	109.0	3.5	108.5	3.3
	0.5	97.1	3.5	93.7	4.4
	0.05	107.1	2.6	105.4	3.2
water	0.5	99.1	5.5	100.8	5.9
	0.05	90.5	4.2	85.1	4.8
	0.005	97.7	3.7	94.1	3.1
		diclofop-methyl			
soil	1	87.6	4.4	95.5	4.4
	0.25	89.5	2.4	98.1	1.8
	0.05	NR	NR	110.1	3.9
water	0.5	89.7	1.3	90.4	1.5
	0.05	93.1	4.5	93.8	1. 2
	0.005	103.0	4.2	91.5	0.5

[a] NR, not recovered.

Although many studies on the effect of temperature on chiral separation were performed, the mechanisms of the temperature impact on the enantioselectivity were not explicitly explained, especially in what way the temperature alters the enthalpy and entropy change related to the transfer of solutes from the mobile to the stationary phase.

Elution Orders. Combining the chiral HPLC and CD is an important technique for identifying low amounts of enantiomer (*24*). The elution order of 10 chiral pesticides was determined by a JASCO HPLC system with a CD detector, and **Table 3** shows the chromatographic conditions and the results.

Quantitative Analysis Methods Setup. For ethofumesate, benalaxyl, and diclofop-methyl, which had better separations, the HPLC quantitative analysis methods were set up. Validation of the methods included linearity, precision, and LOD. The chromatographic conditions and results are listed in **Table 4**.

Five concentration levels of the standard solution for the three chiral pesticides enantiomers were prepared. The mobile phase was *n*-hexane/IPA. It can be seen from **Table 4** that excellent linearities were obtained with a linear coefficient R exceeding

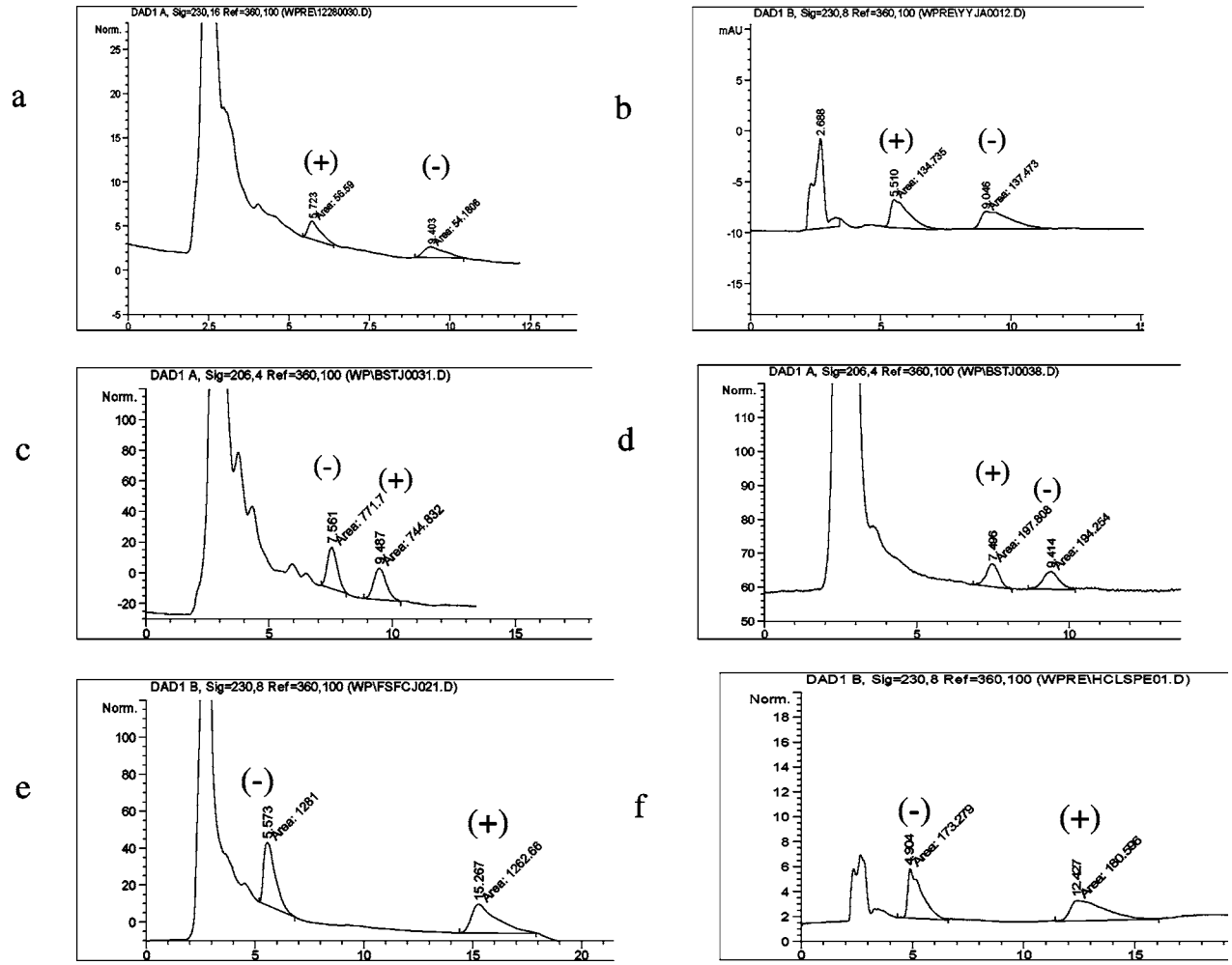

Figure 4. Chromatograms for residual analysis of the enantiomers of ethofumesate, benalaxyl, and diclofop-methyl in soil and water. (**a**) Ethofumesate from soil at 0.1 mg kg^{-1} spiked level, *n*-hexane/IPA 90:10, 230 nm; (**b**) ethofumesate from water at 0.05 mg kg^{-1} level, *n*-hexane/IPA 90:10, 230 nm; (**c**) benalaxyl from soil at 0.25 mg kg^{-1} spiked level, *n*-hexane/IPA 85:15, 206 nm; (**d**) benalaxyl from water at 0.05 mg kg^{-1} spiked level, *n*-hexane/IPA 85:15, 206 nm; (**e**) diclofop-methyl from soil at 1.0 mg kg^{-1} spiked level, *n*-hexane/IPA 90:10, 230 nm; and (**f**) diclofop-methyl from water at 0.05 mg kg^{-1} spiked level, *n*-hexane/ IPA 90:10, 230 nm.

0.99 for all of the enantiomers at a wide concentration range. The LODs were 0.06, 0.13, and 0.05 mg L^{-1} for the single enantiomers of ethofumesate, benalaxyl, and diclofop-methyl, respectively. The precisions (RSD) of the peak area were less than 7.5% for all of the enantiomers calculated based on the injections in triplicate for each concentration. The results showed that the methods were reliable for quantitative enantiomeric analyses of the three chiral pesticides.

Enantiomeric Residual Analysis in Environmental Samples. The residual analysis of the three chiral pesticide enantiomers in soil and water was also performed. **Table 5** shows the recovery and precision obtained by using the above-described extraction procedure to soil and water samples.

Recoveries of the two enantiomers of ethofumesate from soil at three fortification levels (2.5, 0.5, and 0.1 mg L^{-1}) were in the range of 99−113 and 106−122% from water at three levels (0.5, 0.05, and 0.005 mg L^{-1}). Recoveries of single benalaxyl enantiomers at three levels (2.50, 0.25, and 0.025 mg kg^{-1}) from soil were in the range of 94−109%, with RSD < 4.44%, and those from the water sample at three levels (0.5, 0.005, and 0.05 mg L^{-1}) were 85−101%, with RSD < 5.89%. Recoveries of the first eluted enantiomer of diclofop-methyl were 88 and 90% from soil at 1.0 and 0.25 mg L^{-1} levels, respectively, but were not recovered from soil at the 0.05 mg L^{-1} level. The recoveries of the second eluted enantiomer were in the range of 96−110% at the three levels. The RSDs were below 10%. **Figure 4** shows the chromatograms for the residual analysis of the enantiomers in soil and water samples.

Acetone was selected as the solvent for extraction of the enantiomers in soil because of its effectiveness and the solubility in water. The extraction procedure did not require dry soil. The soil samples could be analyzed immediately after sampling without dryness when studying the enantiomeric residual or degradation behaviors in soil, avoiding the changes during the dryness procedure.

Conclusion. Ten chiral pesticides were separated on the CDMPC CSP in an HPLC system in this manuscript, in which eight achieved complete resolutions and two obtained partial resolutions. The effects of alcoholic modifiers and temperature on the resolutions were studied. The elution orders of the chiral pesticides on the CSP were identified by a CD detector. The quantitative analysis methods for the enantiomers of ethofumesate, benalaxyl, and diclofop-methyl were set up, and the residual analysis methods in environmental samples were also developed. The work in this manuscript allows both the determination of the optical purity of the technical product and the further research on enantiomeric behaviors in the environment.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; CDMPC, cellulose-tris(3,5-dimethylphenylcarbamate); CSP, chiral stationary phase; CD, circular dichroism; GC, gas chromatography; LOD, limit of detection; RSD, relative standard deviation; IPA, 2-propanol.

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Received for review October 24, 2005. Revised manuscript received January 15, 2006. Accepted January 19, 2006. This work was supported by National Natural Science Foundation of China (20377052).

JF052631O