Direct Enantioseparation of Nitrogen-Heterocyclic Pesticides on Cellulose-Based Chiral Column by High-Performance Liquid Chromatography

TINGTING CHAI,[1,2†] WENWEN YANG,[1,2†] JING QIU,[2] AND SHICONG HOU[1*]

[1]*College of Science, China Agricultural University, Beijing, China*

[2]*Institute of Quality Standards & Testing Technology for Agro-Products, Key Laboratory of Agro-product Quality and Safety, Chinese Academy of Agricultural Sciences, Beijing, China*

ABSTRACT The enantiomeric separation of eight pesticides including bitertanol (**1**), diclobutrazol (**2**), fenbuconazole (**3**), triticonazole (**4**), imazalil (**5**), triapenthenol (**6**), ancymidol (**7**), and carfentrazone-ethyl (**8**) was achieved, using normal-phase high-performance liquid chromatography on two cellulosed-based chiral columns. The effects of isopropanol composition from 2% to 30% in the mobile phase and column temperature from 5 to 40 °C were investigated. Satisfactory resolutions were obtained for bitertanol **(1)**, triticonazole **(4)**, imazalil **(5)** with the (+)-enantiomer eluted first and fenbuconazole **(3)** with the (—)-enantiomer eluted first on Lux Cellulose-2 and Lux Cellulose-3. (+)-Enantiomers of diclobutrazol **(2)** and triapenthenol **(6)** were first eluted on Lux Cellulose-2. (—)-Carfentrazone-ethyl **(8)** were eluted first on Lux Cellulose-2 and Lux Cellulose-3 with incomplete separation. Reversed elution orders were obtained for ancymidol (7). (+)-Ancymidol was first eluted on Lux Cellulose-2 while on Lux Cellulose-3 (—)-ancymidol was first eluted. The results of the elution order at different column temperatures suggested that column temperature did not affect the optical signals of the enantiomers. These results will be helpful to prepare and analyze individual enantiomers of chiral pesticides. *Chirality 27:32–38, 2015.*

KEY WORDS: enantioseparation; chiral pesticide; chiral stationary phase; cellulose; HPLC

INTRODUCTION

Chirality has been a common property in the field of commercial agrochemical compounds.[1] A large number of pesticides contain stereogenic centers and are introduced into markets in the form of racemates. However, their enantiomers often have distinct behaviors in biological and environmental systems.[2] Thus, the optically pure forms of chiral pesticides are required to improve application efficiency, avoid possible side effects caused by one enantiomer, and study enantioselective toxicity, bioactivity, and environmental behaviors. Now many analytical technologies have been applied to detect chiral pesticides such as high-performance liquid chromatography (HPLC),[3] HPLC-tandem mass spectrometry (LC-MS/MS),[4] gas chromatography-mass spectrometry (GC-MS),[5] and supercritical fluid chromatography (SFC).[6] HPLC with chiral stationary phase (CSP) is one of the most useful methods for enantioseparation at both the analytical and preparative scales. Cellulose-*tris*(3,5-dimethylphenylcarbamate) (CDMPC)[7,8] and amylose-*tris*(3,5-dimethylphenyl-carbamate) (ADMPC)[9,10] belong to polysaccharide-based CSPs, which were mostly used over the past three decades because they exhibit excellent separation toward a large variety of chiral compounds.[11,12]

In this report, two cellulose-based chiral columns including Lux Cellulose-2 and Lux Cellulose-3 were used to separate eight pesticides, bitertanol **(1)**, diclobutrazol **(2)**, fenbuconazole **(3)**, triticonazole **(4)**, imazalil **(5)**, triapenthenol **(6)**, ancymidol **(7)**, and carfentrazone-ethyl **(8)** (Figure 1). The influence of column temperature and mobile phase composition on resolution were investigated. In addition, the enantiomeric elution orders on two chiral columns were detected by optical rotation detector (OR) in different separation conditions.

EXPERIMENTAL

Chemicals and Reagents

Racemic bitertanol **(1)**, diclobutrazol **(2)**, fenbuconazole **(3)**, triticonazole **(4)**, imazalil **(5)**, triapenthenol **(6)**, ancymidol **(7)**, carfentrazone-ethyl **(8)** were purchased from Dr. Ehrenstorfer (Germany) with purity of >95%. Stock solutions of all eight compounds were prepared at 1000 mg/L in 2-propanol (IPA). 1,3,5-Tri-tert-butylbenzene (100 mg/L) was used to determine the void time (t_0). *n*-hexane and IPA (HPLC grade) were obtained from Fisher Scientific (UK).

Instrumentation

Chromatographic separation was carried out on an Agilent 1200 series HPLC equipped with a G1322A degasser, G1311A QuatPump, G1316B column compartment, G1315C diode array detector, G1329A autosampler, and a 20-μl sample loop (Wilmington, DE). UV signals were acquired and manipulated using an Agilent Chemstation. The enantiomeric optical rotations of chiral analytes were determined at 426 nm using a CHIRALYSER-MP optical rotation detector produced by IBZMesstechnik (Germany) and provided by the Beijing Separation Science & Technology Development Co. (Beijing, China). Optical signals were acquired and processed using an Agilent Chemstation with signal transformation using an Agilent 35900E A/D converter.

Chromatographic Conditions

Enantioseparation was obtained on chiral columns including Lux Cellulose-2 (cellulose *tris*-(3-chloro-4-methylphenylcarbamate) (CCMPC) and Lux Cellulose-3 (cellulose *tris*-(4-methylbenzoate) (CTMB),

*Correspondence to: Shicong Hou; Professor; College of Science, China Agricultural University, Beijing 100193, China. E-mail: houshc@cau.edu.cn

†The first two authors contributed equally to this work.

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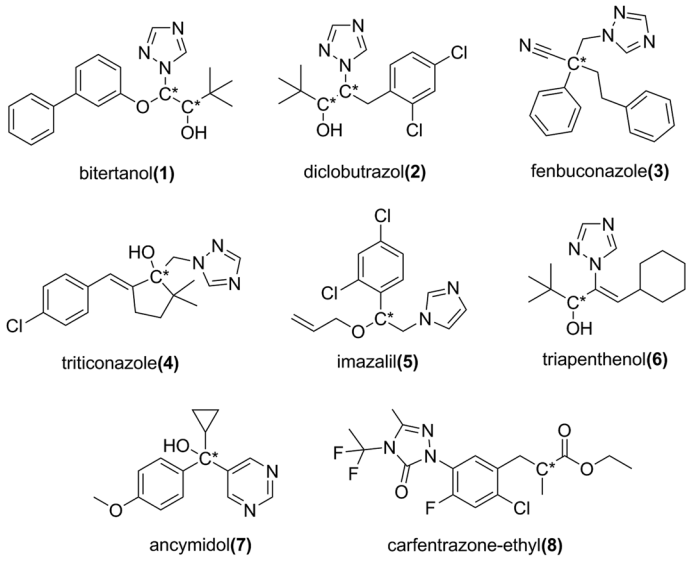

Fig. 1. Molecular structures of eight chiral pesticides.

manufactured by Phenomenex (Torrance, CA), and obtained from Guangzhou FLM Scientific Instrument Co. The structures of chiral columns are shown in Figure 2. The columns were 250 mm × 4.6 mm (i.d., inner diameter) and packed with 5-μm particles. The mobile phases were composed of different proportions of *n*-hexane and IPA. The flow rate was 0.8 mL/min. The column temperature was in the range of 5–40 °C. The UV detection wavelength for bitertanol **(1)** and diclobutrazol **(2)** was set at 210 nm, and for other target analytes at 220 nm. The injection volume was 10 μL.

Chromatographic parameters were calculated using the following equations. The retention factor was calculated as:

$$k' = (t_R - t_0)/t_0 \quad (1)$$

where t_R is retention time and t_0 is void time. The separation factor was calculated as:

$$\alpha = k_2'/k_1' \quad (2)$$

where k_1' and k_2' are retention factors of the first and second eluting enantiomers, respectively. The resolution factor was calculated as:

$$R_s = 2(t_2 - t_1)/(W_2 + W_1) \quad (3)$$

where W_1 and W_2 are the base peak widths of the first and second eluting enantiomers, respectively.

RESULTS AND DISCUSSION

Enantiomeric Separation on Lux Cellulose-2 and Lux Cellulose-3

The data of enantioseparation both on Lux Cellulose-2 and Lux Cellulose-3 are summarized in Table 1 with different compositions of mobile phase (IPA:hexane). With the decrease of IPA from 30% to 2%, the separation factor (α) increased slightly and better resolution factors (*R*s) were achieved on both chiral columns for bitertanol **(1)**, diclobutrazol **(2)**, fenbuconazole **(3)**, triticonazole **(4)**, imazalil **(5)**, triapenthenol **(6)** and carfentrazone-ethyl **(8)** on Lux Cellulose-2 and Lux Cellulose-3. For example, the *Rs* value of triticonazole **(4)** on Lux Cellulose-2 was 25.20 when the percentage of IPA was 10%, while the *Rs* value of triticonazole **(4)** decreased to 15.25 when the composition of IPA increased to 30%. For triapenthenol **(6)**, incomplete resolution was obtained with 10% of IPA in mobile phase, while complete separation was obtained with 2% of IPA. But ancymidol **(7)** was an exception, as with the decrease of IPA from 20% to 5%,the *Rs* value decreased from 1.01 to 0.68.

As shown in Figure 1, all eight pesticides contain a nitrogen-heterocyclic ring. Theoretically, bitertanol **(1)** and diclobutrazol **(2)** have four enantiomers for the existing two chiral carbons. However, in a previous study,[13] two peaks of bitertanol **(1)** on Lux Cellulose-1 could be separated with *Rs* 1.5 when acetonitrile was used as an organic modifier combined with an aqueous solution. This separation also can be obtained by capillary electrophoresis (CE) when an organic solvent such as methanol or acetonitrile is added.[14] Fenbuconazole **(3)** could be separated with excellent resolution on Lux Cellulose-2 in both normal-phase and reverse-phase HPLC systems, which suggested that the change of mobile phases from IPA to ethanol or acetonitrile did not

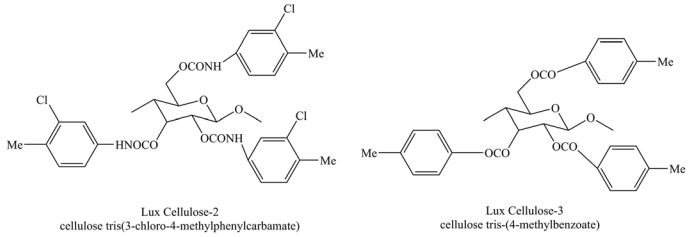

Fig. 2. Structures of CCMPC and CTMB.

TABLE 1. Stereoselective separations of chiral pesticides on Lux Cellulose-2 and Lux Cellulose-3 with different contents of IPA

		Lux Cellulose-2						Lux Cellulose-3					
Pesticides	Wavelength (nm)	Content of IPA(%)	k_1	k_2	α	*R*s	Elution order	Content of IPA(%)	k_1	k_2	α	*R*s	Elution order
bitertanol (1)	210	5	9.41	14.42	1.53	7.75	(+)/(-)	5	6.79	13.85	2.04	12.13	(+)/(-)
		10	3.95	5.14	1.30	4.04		10	3.05	5.79	1.90	7.09	
		20	1.69	1.91	1.13	1.66		20	1.09	1.97	1.81	3.92	
diclobutrazol (2)	220	5	3.34	3.96	1.19	2.52	(+)/(-)	5	—	—	—	—	
		10	1.46	1.68	1.15	2.13		10	—	—	—	—	
		20	0.66	0.76	1.15	1.63		20	—	—	—	—	
fenbuconazole (3)	220	20	10.11	12.69	1.26	3.43	(-)/(+)	20	7.18	15.01	2.09	6.86	(-)/(+)
		30	10.70	12.00	1.12	1.95		30	4.07	8.51	2.09	6.71	
triticonazole (4)	220	10	9.86	35.71	3.62	25.20	(+)/(-)	5	2.86	6.04	2.11	8.49	(+)/(-)
		20	3.94	14.41	3.66	24.11		10	1.22	2.41	1.98	5.19	
		30	2.41	8.57	3.56	15.25		20	0.53	0.98	1.86	3.49	
imazalil (5)	220							5	3.89	8.58	2.20	12.68	(+)/(-)
		10	10.18	13.36	1.31	4.75	(+)/(-)	10	1.80	3.91	2.18	10.79	
		20	4.28	5.65	1.32	2.93		20	0.80	1.71	2.13	7.31	
triapenthenol (6)	220	2	8.17	9.48	1.16	2.62	(+)/(-)	5	—	—	—	—	
		5	2.60	2.93	1.12	1.62		10	—	—	—	—	
		10	1.16	1.28	1.10	1.10		20	—	—	—	—	
ancymidol (7)	220							5	9.10	9.25	1.02	0.68	(-)/(+)
		10	5.29	5.50	1.04	0.86	(+)/(-)	10	3.65	3.77	1.03	0.94	
		20	—	—	—	—		20	1.52	1.60	1.05	1.01	
carfentrazone-ethyl (8)	220	2	3.09	3.30	1.07	1.15	(-)/(+)	5	3.25	3.79	1.17	3.08	(-)/(+)
		10	1.48	1.59	1.07	1.15		10	2.13	2.45	1.15	2.72	
		20	0.98	1.02	1.04	0.80		20	1.34	1.54	1.14	2.20	

affect the enantioselective separation of fenbuconazole **(3)** on Lux Cellulose-2.[1] However, it can be partially separated on Lux Cellulose-1[15] by reverse-phase HPLC. This indicates that Lux Cellulose-2 and Lux Cellulose-3 showed better chiral discriminability for fenbuconazole **(3)** than Lux Cellulose-1. Imazalil **(5)** was separated on amylose tris-(S)-1-phenylethylcarbamate (AS)[16] with *Rs* in the range of 1.08-1.12, using normal-phase HPLC. The enantioselective separation of imazalil **(5)** was investigated on various types of CSPs including cellulose tribenzoate (CTB), CTMB with the maximal resolution of 4.83, cellulose triphenylcarbamate (CTPC), and cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC).[17] In our study, imazalil **(5)** was completely separated on Lux Cellulose-3, identical to CTMB, with a maximal resolution of 12.68 and on Lux Cellulose-2, identical to CCMPC, with a maximal resolution of 4.75. Comparatively, imazalil **(5)** got better separation on CTMB, compared to other types of CSPs. Carfentrazone-ethyl **(8)** was partially separated on CDMPC and on Lux Cellulose-2 with IPA as mobile phase,[18] but satisfactory separation on Lux Cellulose-3.

In our study, different separation capacities for the eight analytes on Lux Cellulose-2 and Lux Cellulose-3 were exhibited differently. Under a suitable percentage of IPA, all chiral analytes could obtain baseline separation ($Rs > 1.5$) except ancymidol **(7)** and carfentrazone-ethyl **(8)** on Lux Cellulose-2**,** and for diclobutrazol **(2),** triapenthenol **(6),** and andancymidol **(7)** on Lux Cellulose-3**.** Generally, Lux Cellulose-3 with a substituted group of 4-methylbenzoate on cellulose-based chiral stationary showed better chiral discriminability for bitertanol **(1)**, fenbuconazole **(3)**, imazalil **(5),** and carfentrazone-ethyl **(8)** than Lux Cellulose-2 with 3-chloro-4-methylphenylcarbamate. But for diclobutrazol **(2)**, triticonazole **(4)**, triapenthenol **(6),** Lux Cellulose-2 showed better chiral discriminability than Lux Cellulose-3. The differences of the substituted groups on cellulose-based chiral stationary might result in the change of enantiomer–CSP interactions and recognition mechanisms. In the enantiomer–CSP interactions and recognition mechanisms, enantiomers with different stereochemistries were inserted into chiral grooves of CSPs in the process of solute-CSP complexes formation, and then enantioseparation on CSPs occurs,[19] OH groups on chiral centers are available to participate in hydrogen bonding with the C=O group of the CSP. Additionally, charge transfer π–π interactions between the CSP and the aromatic rings of the racemates can also provide stabilization of the solute–CSP complexes[20].

Effects of Column Temperature on Enantioseparation

The effects of column temperatures from 5 to 40 °C on Lux Cellulose-2 and Lux Cellulose-3 were investigated and the results are summarized in Table 2. The column temperature was found to influence the enantiomer–CSPs interactions. At rising temperature, k_1 and k_2 gradually decreased on Lux Cellulose-2 and Lux Cellulose-3 for all eight analytes. However, α and *R*s values did not follow consistent trends. On Lux Cellulose-2, α and *R*s consistently declined for bitertanol **(1)**, diclobutrazol **(2)**, triticonazole **(4)**, imazalil **(5)**, and carfentrazone-ethyl **(8)** with an increase of column temperature. But for fenbuconazole **(3)**, triapenthenol **(6)**, and ancymidol **(7)**, α and *R*s increased with temperature. On Lux Cellulose-3, α generally decreased with increasing temperature, while *R*s increased for all the analytes except for ancymidol **(7)**.

The van't Hoff equations[21] as follows were used to calculate the thermodynamics parameters shown in

TABLE 2. Effects of column temperatures on separation of chiral pesticides on Lux Cellulose-2 and Lux Cellulose-3

Pesticides	Temperature	Lux Cellulose-2					Lux Cellulose-3				
	(°C)	Hexane/IPA	k_1	k_2	α	*R*s	Hexane/IPA	k_1	k_2	α	*R*s
bitertanol **(1)**	5	95/5	10.76	17.09	1.59	9.23	95/5	8.07	16.50	2.04	7.21
	10		10.17	15.97	1.57	8.11		7.52	15.48	2.06	8.57
	20		9.41	14.42	1.53	7.75		6.84	13.93	2.04	12.13
	30		8.73	13.16	1.51	7.48		6.22	12.53	2.01	13.76
	40		7.99	11.96	1.50	7.34		5.73	11.24	1.96	15.70
diclobutrazol **(2)**	5	95/5	3.29	3.90	1.19	2.52		—	—	—	—
	10		3.28	4.11	1.26	3.15		—	—	—	—
	20		3.12	3.67	1.17	2.49		—	—	—	—
	30		3.01	3.53	1.17	2.56		—	—	—	—
	40		2.92	3.42	1.17	2.52		—	—	—	—
fenbuconazole **(3)**	5	80/20	26.02	28.46	1.09	1.76	70/30	10.53	26.71	2.54	3.80
	10		23.15	25.65	1.11	2.86		8.38	19.07	2.28	4.70
	20		10.11	12.69	1.26	3.43		4.10	8.56	2.09	6.71
	30		14.43	16.52	1.14	3.24		3.49	6.46	1.85	8.19
	40		11.32	13.12	1.16	3.38		2.98	4.90	1.65	8.81
triticonazole **(4)**	5	80/20	4.56	17.60	3.86	20.95	95/5	3.51	7.45	2.12	5.64
	10		4.37	16.42	3.76	21.36		3.13	6.78	2.16	6.45
	20		3.94	14.41	3.66	24.11		2.88	6.08	2.11	8.49
	30		3.44	11.55	3.36	20.72		2.67	5.34	2.00	10.06
	40		3.07	9.52	3.11	20.22		2.59	4.94	1.91	11.21
imazalil **(5)**	5	90/10	12.74	17.11	1.34	5.20	95/5	5.95	13.99	2.35	12.36
	10		12.17	16.12	1.32	4.66		4.96	11.59	2.34	11.80
	20		10.18	13.36	1.31	4.75		3.92	8.63	2.20	12.68
	30		9.01	11.61	1.29	3.85		3.71	7.48	2.02	13.71
	40		8.34	9.82	1.18	2.38		2.33	4.97	2.13	19.72
triapenthenol **(6)**	5	95/5	2.79	3.10	1.11	1.39		—	—	—	—
	10		2.70	3.02	1.12	1.52		—	—	—	—
	20		2.60	2.93	1.12	1.62		—	—	—	—
	30		2.47	2.82	1.14	1.83		—	—	—	—
	40		2.27	2.65	1.16	2.00		—	—	—	—
ancymidol **(7)**	5	95/5	16.46	16.81	1.02	0.58	95/5	12.03	12.67	1.05	1.29
	10		15.43	15.76	1.02	0.58		10.41	10.80	1.04	1.08
	20		12.66	13.50	1.07	1.52		9.15	9.31	1.02	0.68

Table 3 to better understand the thermodynamic effects on enantioseparation.

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

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

where ΔH° and ΔS° are the changes of standard enthalpy and entropy for the process of analyte from the mobile phase to the stationary phase. ΔΔH° and ΔΔS° are the differences of ΔH_2°-ΔH_1° and ΔS_2°-ΔS_1°, respectively. R is the gas constant, T is absolute temperature, Φ is the phase ratio. If plots of lnk versus 1/T are linear, -ΔH°/R and ΔS°/R+lnΦ are the slope and intercept. For linear plots of lnα versus 1/T, the slope and intercept are -ΔΔH°/R and ΔΔS°/R, respectively.

The values of ΔH° were negative and between –0.0050 and –0.0412 kJ/mol both on Lux Cellulose-2 and Lux Cellulose-3, which suggested that an enthalpy-driven process happened with the transfer of the enantiomers from mobile phase to the stationary phase. The ΔH_2° values were more negative than ΔH_1° values on Lux Cellulose-3 for bitertanol **(1)**, fenbuconazole **(3)**, triticonazole **(4)**, imazalil **(5),** and carfentrazone-ethyl **(8)** and on Lux Cellulose-2 for bitertanol **(1)**, diclobutrazol **(2)**, triticonazole **(4)**, imazalil **(5),** and carfentrazone-ethyl **(8)**, which meant a weaker combination of first-eluted enantiomer with CSPs. The positive ΔΔH° values of fenbuconazole **(3)**, triapenthenol **(6)**, and ancymidol **(7)** on Lux Cellulose-2 suggested that a higher temperature was helpful to get better resolution. On the other hand, negative ΔΔH° values of other analytes meant a lower temperature was helpful to get better resolution. The ΔS° values of all target compounds were positive on Lux Cellulose-2 and Lux Cellulose-3. The ΔΔS° values of eight compounds were positive, which indicated that the enantioselectivity was not an enthalpy-driven course. However, most compounds cannot get the linear relations between ln(α) or ln(*k*) and 1/T both on Lux Cellulose-2 and Lux Cellulose-3. The effects of temperature on the resolution might depend not only on the structure of the CSPs but also on that of the analytes.

Elution Order

The optical rotation for all the enantiomers of target pesticides were identified with an optical rotation detector at 426 nm in different mobile phase systems. The results of the elution order are summarized in Table 1. Although triapenthenol **(6),** ancymidol **(7),** and carfentrazone-ethyl **(8)** could not be significantly separated on two chiral

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TABLE 3. Van't hoff equations and thermodynamic parameters

Pesticides	Columns	$\ln k^{a}=-\Delta H°/RT+\Delta S°/R$	R_1^2	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/mol)	$\ln \alpha=-\Delta\Delta H°/RT+\Delta\Delta S°/R$	R_2^2	$\Delta\Delta H°$ (kJ/mol)	$\Delta\Delta S°$ (J/mol)
Lux Cellulose-2	bitertanol **(1)**	$\ln k_1 = 1.4455/T + 2.1180$	0.7760	-0.0120	17.6091	$\ln \alpha = 0.3261/T + 0.4046$	0.8464	-0.0027	3.3638
		$\ln k_2 = 1.7716/T + 2.5226$	0.7926	-0.0147	20.9729				
	diclobutrazol **(2)**	$\ln k_1 = 0.6024/T + 1.0891$	0.7066	-0.0050	9.0548	$\ln \alpha = 0.1285/T + 0.1645$	0.0971	-0.0011	1.3677
		$\ln k_2 = 0.731/T + 1.2535$	0.4989	-0.0061	10.4216				
	fenbuconazole **(3)**	$\ln k_1 = 4.9509/T + 2.3579$	0.7179	-0.0412	19.6036	$\ln \alpha = -0.4593/T + 0.1779$	0.3791	0.0038	1.4791
		$\ln k_2 = 4.4915/T + 2.5358$	0.7474	-0.0373	21.0826				
	triticonazole **(4)**	$\ln k_1 = 1.9215/T + 1.187$	0.6992	-0.0160	9.8687	$\ln \alpha = 0.9607/T + 1.1846$	0.6073	-0.0080	9.8488
		$\ln k_2 = 2.8822/T + 2.3716$	0.6698	-0.0240	19.7175				
	imazalil **(5)**	$\ln k_1 = 2.2521/T + 2.1526$	0.7811	-0.0187	17.8967	$\ln \alpha = 0.4745/T + 0.2143$	0.428	-0.0039	1.7817
		$\ln k_2 = 2.7267/T + 2.3669$	0.7355	-0.0227	19.6784				
	triapenthenol **(6)**	$\ln k_1 = 0.9172/T + 0.8654$	0.6719	-0.0076	7.1949	$\ln \alpha = -0.2149/T + 0.141$	0.6313	0.0018	1.1723
		$\ln k_2 = 0.7023/T + 1.0064$	0.6828	-0.0058	8.3672				
	ancymidol **(7)**	$\ln k_1 = 2.4989/T + 2.3615$	0.8029	-0.0208	19.6335	$\ln \alpha = -0.3137/T + 0.0746$	0.7568	0.0026	0.6202
		$\ln k_2 = 2.1852/T + 2.4361$	0.7999	-0.0182	20.2537				
	carfentrazone-ethyl **(8)**	$\ln k_1 = 1.9231/T + 1.294$	0.7876	-0.0160	10.7583	$\ln \alpha = 0.1887/T + 0.0697$	0.772	-0.0016	0.5795
		$\ln k_2 = 2.1118/T + 1.3637$	0.7875	-0.0176	11.3378				
Lux Cellulose-3	bitertanol **(1)**	$\ln k_1 = 1.7217/T + 1.7799$	0.8082	-0.0143	14.7981	$\ln \alpha = 0.1564/T + 0.6919$	0.3558	-0.0013	5.7525
		$\ln k_2 = 1.8781/T + 2.4717$	0.7636	-0.0156	20.5497				
	diclobutrazol **(2)**								
	fenbuconazole **(3)**	$\ln k_1 = 7.2154/T + 1.0569$	0.8679	-0.0600	8.7871	$\ln \alpha = 2.0983/T + 0.5497$	0.7997	-0.0174	4.5702
		$\ln k_2 = 9.3137/T + 1.6066$	0.869	-0.0774	13.3573				
	triticonazole **(4)**	$\ln k_1 = 1.6563/T + 0.942$	0.9358	-0.0138	7.8318	$\ln \alpha = 0.4547/T + 0.6855$	0.4081	-0.0038	5.6992
		$\ln k_2 = 2.111/T + 1.6275$	0.824	-0.0176	13.5310				
	imazalil **(5)**	$\ln k_1 = 4.1066/T + 1.046$	0.7025	-0.0341	8.6964	$\ln \alpha = 0.7147/T + 0.7321$	0.6468	-0.0059	6.0867
		$\ln k_2 = 4.8213/T + 1.7781$	0.7548	-0.0401	14.7831				
	triapenthenol **(6)**								
	ancymidol **(7)**								
	carfentrazone-ethyl **(8)**	$\ln k_1 = 3.1547/T + 1.2789$	0.206	-0.0262	10.6328	$\ln \alpha = 0.4109/T + 0.1092$	0.9567	-0.0034	0.9079
		$\ln k_2 = 3.5655/T + 1.3882$	0.2449	-0.0296	11.5415				

[a]k_1 and k_2 were respectively calculated for first- and second-eluted enantiomers of analyte.

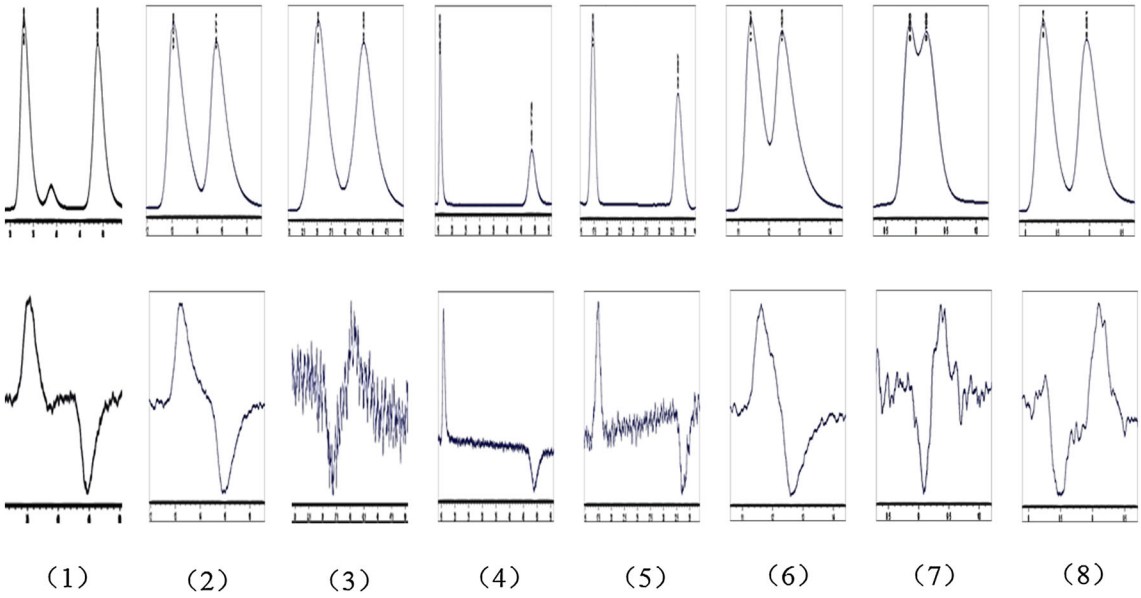

Fig. 3. Typical UV and OR chromatograms of bitertanol **(1)**, diclobutrazol **(2)**, fenbuconazole **(3)**, triticonazole **(4)**, imazalil **(5)**, triapenthenol **(6)**, ancymidol **(7)**, and carfentrazone-ethyl **(8)**. Chromatographic conditions: hexane/IPA at 95/5 for **(1)**, **(2)**, **(6)**, and 80/20 for **(3)** and **(4)** on Lux Cellulose-2, at 95/5 for **(5)** and 80/20 for **(7)** and **(8)** on Lux Cellulose-3. All column temperatures were 20 °C.

columns with a UV detector, the elution orders could be obtained by an optical rotation detector (shown in Figure 3). The right rotation (+)-enantiomer was first eluted from Lux Cellulose-2 for bitertanol **(1)**, diclobutrazol **(2)**, triticonazole **(4)**, imazalil **(5),** and triapenthenol **(6)**. But for fenbuconazole **(3)** and carfentrazone-ethyl **(8),** the left rotation (—)-enantiomer was first eluted. The first elution of ancymidol **(7)** on Lux Cellulose-2 was (+)-ancymidol, while on Lux Cellulose-3 it was (—)-ancymidol. The column temperature from 5 to 40 °C and the percentage of IPA from 2% to 30% did not affect the stereoisomeric rotation for all chiral analytes.

CONCLUSION

Eight chiral compounds were successfully separated with satisfactory resolution on Lux Cellulose-2 and Lux Cellulose-3, using NP-HPLC with hexane/IPA as the mobile phase. Generally, with a decrease of IPA, better resolutions were achieved on both chiral columns. Although most compounds cannot achieve linear relations between ln (α) or ln (k) and 1/T on Lux Cellulose-2 and Lux Cellulose-3 when the column temperature was in the range of 5 to 40 °C, α and Rs were changed to some extent. The results from OR showed that the temperature changes did not reverse the stereoisomeric optical signals of all pesticides, but the signals of some pesticides would change on different columns. This work will be helpful to easily prepare single enantiomers from racemic mixtures, and to establish effective analytical methods for future study on the stereoselective behaviors of chiral pesticides in the environment.

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