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REGULAR ARTICLE

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Enantiomeric separation of type I and type II pyrethroid insecticides with different chiral stationary phases by reversed-phase high-performance liquid chromatography

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Abstract

The enantiomeric separation of type I (bifenthrin, BF) and type II (lambda-cyhalothrin, LCT) pyrethroid insecticides on Lux Cellulose-1, Lux Cellulose-3, and Chiralpak IC chiral columns was investigated by reversed-phase high-performance liquid chromatography. Methanol/water or acetonitrile/water was used as mobile phase at a flow rate of 0.8 mL/min. The effects of chiral stationary phase, mobile phase composition, column temperature, and thermodynamic parameters on enantiomer separation were carefully studied. Bifenthrin got a partial separation on Lux Cellulose-1 column and baseline separation on Lux Cellulose-3 column, while LCT enantiomers could be completely separated on both Lux Cellulose-1 and Lux Cellulose-3 columns. Chiralpak IC provided no separation ability for both BF and LCT. Retention factor (k) and selectivity factor (α) decreased with the column temperature increasing from 10°C to 40°C for both BF and LCT enantiomers. Thermodynamic parameters including ΔH and ΔS were also calculated, and the maximum R_s were not always obtained at lowest temperature. Furthermore, the quantitative analysis methods for BF and LCT enantiomers in soil and water were also established. Such results provide a new approach for pyrethroid separation under reversed-phase condition and contribute to environmental risk assessment of pyrethroids at enantiomer level.

KEYWORDS

bifenthrin, enantiomeric separation, enantiomers, HPLC, lambda-cyhalothrin

1 | INTRODUCTION

Pyrethroids are a class of insecticides synthesized based on natural pyrethrins, the insecticidal compound in certain species of *Chrysanthemum*.[1] Considering their outstanding insecticidal potency and relatively low toxicity, pyrethroids have been widely used in agriculture and households over the past several decades.[2] The worldwide usage of pyrethroids has been anticipated to be further increased with the ban or restriction on organophosphorus and organochlorine pesticide in many countries.[3,4] Based on chemical structures, pyrethroids are classified into type I and type II categories. Type I pyrethroids do not have α-cyano group on the phenoxybenzyl moiety, while type II pyrethroids have α-cyano group on the phenoxybenzyl moiety to improve their photostability.[5]

Structurally, pyrethroids contain 1 to 3 asymmetric carbon atoms and result in 1 to 4 pairs of enantiomers, which generally exhibit similar physicochemical and chemical properties in nonchiral environment and different bioactivities in organisms because of different interaction capabilities between enantiomers and naturally chiral biomolecules.[6-8] The enantioselective accumulation, degradation, metabolism, and toxicity of pyrethroid enantiomers have been widely studied in recent years.[9,10]

Bifenthrin[BF, 2-methylbiphenyl-3-ylmethyl (1RS,3RS)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate, 82657-04-3] and lambda-cyhalothrin [LCT, (*RS*)-α-cyano-3-phenoxybenzyl (1RS,3RS)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate, 91465-08-6] are 2 of the most popular pyrethroids used to control insect pests and acarids in agriculture (Figure 1). In chemical structure, BF and LCT are classified into type I and type II pyrethroids, respectively. Commercial BF consists of 2 enantiomers (1S-cis-BF and 1R-cis-BF), and LCT only contains 1 of 4 pairs of enantiomers ([Z]-1R-cis-αS and [Z]-1S-cis-αR). The bioactivities of BF and LCT enantiomers are significantly different in environment and organisms. For example, the LC_{50} of 1R-cis-BF are 22- and 17-fold more toxic than 1S-cis-BF to *Daphnia magna* and *Ceriodaphnia dubia* individually.[9,11] After BF application, the more toxic enantiomer 1R-cis-BF is also more persistent in soil, sediments, and water.[12,13] For LCT, the toxicity of (−)-LCT is about 162-fold of (+)-LCT to zebrafish in 96-hour acute toxicity test.[14] Therefore, it is necessary to investigate the environmental behaviors of chiral pyrethroid pesticide at enantiomer level.

Enantiomeric separation of chiral pesticide enantiomers is a key basis for enantiomer-specific behavior research. Generally, reversed-phase high-performance liquid chromatography (HPLC),[15] normal-phase HPLC,[16] gas chromatography,[17] capillary electrophoresis,[18] ultra-performance convergence chromatography,[19] cyclodextrin-modified micellar electrokinetic chromatography,[20] supercritical fluid chromatography,[21] etc. have been developed to separate enantiomers of chiral pesticides.

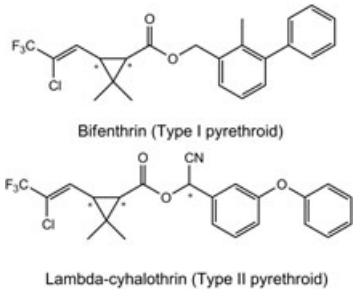

FIGURE 1 Chemical structure of bifenthrin (BF) and lambda-cyhalothrin (LCT). *Asymmetric carbon atom

To date, HPLC combined with different types of chiral stationary phase (CSP) was considered as the most effective and commonly used method for chiral separation.[22] Among the available CSPs, polysaccharide-based CSPs including cellulose-tris-(3,5-dimethylphenylcarbamate), amylose-tris-(3,5-dimethylphenylcarbamate), cellulose-tris-(3,5-dichlorophenylcarbamate), and cellulose-tris-(4-methylbenzoate) were most frequently applied in enantiomer separation. Typically, normal-phase HPLC is more widely applied in chiral separation than reversed-phase HPLC due to better separation efficiency.[23,24] However, reversed-phase HPLC has been rising fast for chiral separation in consideration of lower background signal intensity, easier sample preparation procedures, and better solubility for polar analytes. Furthermore, reversed-phase HPLC is more compatible for electron spray ionization sources than normal-phase HPLC in mass spectrometry detection.[25-27] For instance, hexaconazole, epoxiconazole, tebuconazole, myclobutanil, fenbuconazole, benalaxyl, and metalaxyl were successfully separated on reversed-phase HPLC coupled with tandem mass spectrometry.[28-31] Chiral separation of BF and LCT enantiomers was conducted by gas chromatography,[32] HPLC,[14,33] capillary electrochromatography,[18] and cyclodextrin-modified micellar electrokinetic chromatography.[34] Although a few studies reported the chiral separation of BF and LCT enantiomers by reversed-phase HPLC,[18,35] the complete separation ($R_s > 1.5$) was not achieved under reverse phase condition.

In the context of this study, BF and LCT enantiomers were successfully separated on Lux Cellulose-1, Lux Cellulose-3, and Chiralpak IC chiral columns. The effects of CSPs, mobile phase composition, column temperature, and thermodynamic parameters on resolution were carefully evaluated. Chromatographic parameters including retention factor (k), selectivity factor (α), and resolution factor (R_s) were employed to evaluate the separation efficiency. Furthermore, the quantitative analysis methods for BF and LCT enantiomers in soil and water were also established. Such results shed new light on chiral separation of type I and type II pyrethroids and contribute to environmental risk assessment of pyrethroids at enantiomer level.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Bifenthrin (purity ≥ 97.0%) and LCT (purity ≥ 98.0%) were purchased from J&K Scientific Co. Ltd. (Beijing, China). Stock solutions of BF and LCT were prepared with acetonitrile and stored at 4°C. Water was purified with a Millipore Milli-Q system (Billerica, USA).

Acetonitrile and methanol (HPLC grade) were purchased from Fisher scientific (Beijing, China). All other regents were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China).

2.2 | Apparatus

Reversed-phase chiral HPLC separations were conducted on an Agilent 1260 series HPLC system (Santa Clare, USA), equipped with G1322 degasser, G1311B quatpump, G1329B autosampler with a 100-μL sample loop, G1316A column compartment, and G1315D diode array detector. The signal was collected and analyzed by an Agilent workstation.

2.3 | Chromatographic conditions

Enantiomers of BF and LCT were separated on Lux Cellulose-1 (250 mm × 4.6 mm [i.d.], 5 μm), Lux Cellulose-3 (250 mm × 4.6 mm [i.d.], 5 μm), and Chiralpak IC (250 mm × 4.6 mm [i.d.], 5 μm) chiral columns, respectively. The mobile phase was composed of solvent A (methanol or acetonitrile) and solvent B (water). In each run, the flow rate was 0.8 mL/min, and the 20-μL sample was injected with detection wavelength at 220 nm. Column temperature was changed from 10°C to 40°C. The retention factor ($k = [t - t_0]/t_0$), separation factor ($\alpha = k_2/k_1$), and resolution factor ($R_s = 2[t_2\text{-}t_1]/[w_1 + w_2]$) were calculated.

2.4 | Extraction of bifenthrin and lambda-cyhalothrin enantiomers from soil and water

Three spiked levels (0.02, 0.5, and 5 mg kg^{-1}) of BF and LCT standard solution were added to soil (5 g, dried at room temperature), respectively. After that, 25-mL acetonitrile, 2-g anhydrous sodium sulfate, and 1-g sodium chloride were added to 50-mL polypropylene centrifuge tubes. The mixture was shaken in rotary vibrator for 10 minutes at 300 rpm, exposed to ultrasonic vibration for 15 minutes, and then centrifuged at 3500 rpm for 5 minutes. The extraction solvent was filtered through 10 g of anhydrous sodium sulfate for dehydration. The

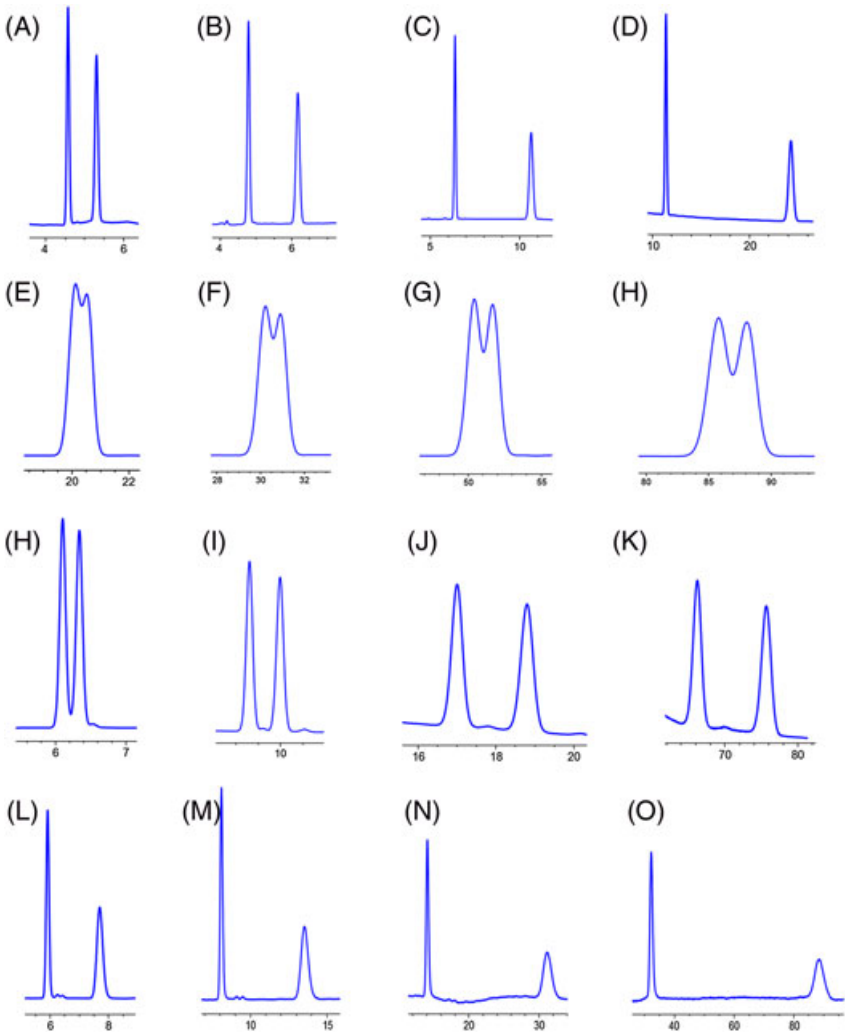

FIGURE 2 Chiral resolution chromatograms of bifenthrin (BF) on Lux Cellulose-3 (ACN/H_2O, A 95/5, B 90/10, C 80/20, and D 70/30), Lux Cellulose-1 (ACN/H_2O, E 75/25, F 70/30, G 65/35, and H 60/40), and lambda-cyhalothrin (LCT) on Lux Cellulose-3 (ACN/H_2O, H 80/20, I 70/30, J 60/40, and K 50/50) and Lux Cellulose-3 (MEOH/H_2O, L 100/0, M 95/5, N 90/10, and O 85/15)

residue soil was extracted by another 25-mL acetonitrile, and all the extraction steps were the same as previous procedures. As for water sample, 25-mL ethyl acetate and 1-g sodium chloride were added, and other steps were the same as soil. The filtered solvent was combined into a pear-bottomed flask and evaporated to near dryness by using a rotary vacuum evaporator at 40°C and reconstituted in 1.0-mL acetonitrile for chiral HPLC analysis.

2.5 | Method validation

The performance of developed method was evaluated based on parameters including linearity, accuracy,

TABLE 1 Enantiomeric separation results of bifenthrin (BF) and lambda-cyhalothrin (LCT) enantiomers on 3 chiral columns using MEOH/H_2O or ACN/H_2O as mobile phase

Compound	Mobile Phase	Ratio (vol/vol)	*k*1	*k*2	*α*	*Rs*
BF	Lux Cellulose-1 MEOH/H_2O	95/5	2.08	2.08	1.00	0.00
		90/10	5.08	5.15	1.01	0.24
		85/15	13.47	13.67	1.01	0.25
BF	Lux Cellulose-1 ACN/H_2O	90/10	1.01	1.01	1.00	0.00
		75/25	3.88	3.98	1.02	0.45
		70/30	6.15	6.31	1.03	0.47
		65/35	10.51	10.80	1.03	0.50
		60/40	17.98	18.47	1.03	0.54
BF	Lux Cellulose-3 MEOH/H_2O	100/0	0.59	1.68	2.86	6.18
		95/5	1.60	4.95	3.09	8.88
		90/10	4.14	13.53	3.26	8.91
BF	Lux Cellulose-3 ACN/H_2O	100/0	0.24	0.34	1.40	1.34
		95/5	0.15	0.33	2.25	2.47
		90/10	0.14	0.47	3.29	3.49
		80/20	0.53	1.55	2.93	7.64
		70/30	1.89	5.14	2.73	10.08
		55/45	4.99	13.97	2.80	13.30
BF	Chiralpak IC MEOH/H_2O	90/10	0.68	0.68	1.00	—
		80/20	4.10	4.10	1.00	—
BF	Chiralpak IC ACN/H_2O	90/10	0.23	0.23	1.00	—
		60/40	5.65	5.65	1.00	—
LCT	Lux Cellulose-1 MEOH/H_2O	100/0	0.50	0.55	1.10	0.36
		95/5	1.14	1.32	1.16	1.02
		90/10	2.91	3.43	1.18	1.47
		85/15	7.39	8.84	1.20	2.03
LCT	Lux Cellulose-1 ACN/H_2O	100/0	0.22	0.24	1.10	0.44
		90/10	0.63	0.70	1.11	0.80
		80/20	2.05	2.26	1.10	1.26
		70/30	6.00	6.64	1.11	1.61
		60/40	18.41	20.44	1.11	1.96
LCT	Lux Cellulose-3 MEOH/H_2O	100/0	0.30	0.69	2.31	4.60
		95/5	0.77	1.96	2.54	4.27
		90/10	2.07	5.79	2.79	6.47
		85/15	5.98	18.22	3.04	8.29
LCT	Lux Cellulose-3 ACN/H_2O	80/20	0.42	0.48	1.13	0.82
		70/30	1.25	1.41	1.13	1.28
		60/40	3.14	3.58	1.14	1.53
		50/50	14.76	17.02	1.15	2.26
LCT	Chiralpak IC MEOH/H_2O	90/10	1.24	1.24	1.00	—
		80/20	6.59	6.59	1.00	—
LCT	Chiralpak IC ACN/H_2O	70/30	2.03	2.03	1.00	—
		60/40	5.27	5.27	1.00	—

precision, limit of detection (LOD), and limit of quantitation (LOQ). The stock standard solutions were prepared in acetonitrile and diluted to a series of concentrations (0.05, 0.1, 0.5, 1, 2, 5, and 10 mg L^{-1}). Linearity was obtained by linear regression of peak area of enantiomer versus the injected concentration. The LOD for each enantiomer was defined as the concentration that produced a signal-to-noise ratio of 3, while the LOQ was considered as the lowest concentration in calibration curve with acceptable accuracy and precision. The recovery and relative standard deviation (RSD) of triple replicates at 3 spiked concentrations (0.02, 0.5, and 5 mg kg^{-1}) were employed to evaluate the accuracy and precision of the developed method. The stability of BF and LCT stock solution was checked weekly and indicated that those 2 compounds were stable under −20°C condition.

3 | RESULTS AND DISCUSSION

3.1 | Chiral resolutions

The enantiomeric separations of BF and LCT enantiomers on 3 chiral columns were performed under reversed-phase HPLC condition (Figure 2). Methanol/water (MEOH/H_2O) or acetonitrile/water (ACN/H_2O) was used as mobile phase to optimize chromatographic condition at a flow rate of 0.8 mL/min at 20°C. The separation parameters including retention factors (k_1 and k_2), separation factor (α), and resolution factor (R_s) were summarized in Table 1, and $R_s > 1.5$ was considered as complete separation. When MEOH/H_2O was used as mobile phase, BF enantiomers got a partial separation (maximum $R_s = 0.25$) and complete separation (maximum $R_s = 8.91$) on Lux Cellulose-1 and Lux Cellulose-3, respectively. As for LCT, the baseline separations were achieved on both Lux Cellulose-1 and Lux Cellulose-3, and maximum R_s were 2.03 and 8.29 at MEOH/H_2O ratio of 85/15, individually. When ACN/H_2O was used as mobile phase, BF got partial separation on Lux Cellulose-1 with maximum $R_s = 0.54$ and complete separation on Lux Cellulose-3 with maximum $R_s = 13.30$, individually. Lambda-cyhalothrin enantiomers were completely separated on Lux Cellulose-1 and Lux Cellulose-3 columns. The maximum R_s is 1.96 on Lux Cellulose-1 with mobile phase ratio of 60/40 and 2.26 on Lux Cellulose-3 with ratio of 50/50, respectively. Chiralpak IC had no ability to separate both BF and LCT enantiomers when MEOH/H_2O or ACN/H_2O was used as mobile phase.

The separation efficiencies of MEOH/H_2O and ACN/H_2O on Lux Cellulose-1 for both BF and LCT were almost the same. However, MEOH/H_2O exhibited better separation ability than ACN/H_2O on Lux Cellulose-3 for BF, while ACN/H_2O was better for LCT on Lux Cellulose-3, which indicated that methanol and acetonitrile exhibited different separation capacities on the same column and the separation efficiencies were related not only to mobile phase but also CSP. It is reported that hydrogen bonding, π-π, and dipole-dipole interactions were main forces for the chiral resolution.[36] In our study, methanol is a protonic solvent, which could donate and accept proton in hydrogen-bond formation. However, acetonitrile is just a weak proton acceptor in the process of hydrogen-bond formation. Therefore, the different separation efficiencies between methanol and acetonitrile may be caused by the different hydrogen-bond interaction between mobile phase and analyte. In general, a lower ratio of methanol or acetonitrile in mobile phase leads to a higher resolution

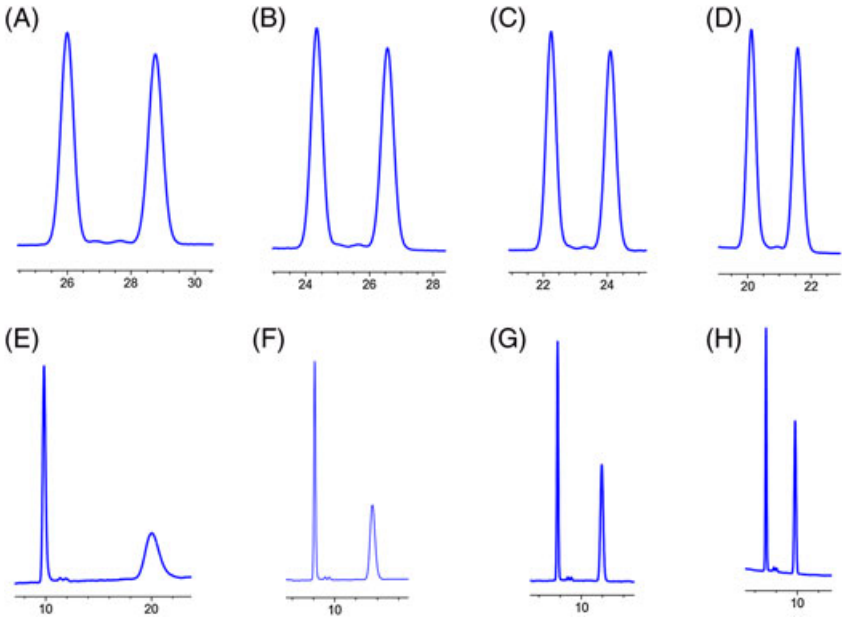

FIGURE 3 The effects of temperature on lambda-cyhalothrin (LCT) enantiomer separation with Lux cellulose-1 column (ACN/H_2O [70/30], A 10°C, B 20°C, C 30°C, and D 40°C) and Lux cellulose-1 column (MEOH/H_2O [95/5], E 10°C, F 20°C, G 30°C, and H 40°C)

TABLE 2 Effects of temperature on bifenthrin (BF) and lambda-cyhalothrin (LCT) separation with Lux Cellulose-1 and Lux Cellulose-3 chiral columns

Compound	Mobile Phase (vol/vol)	Temperature (°C)	*k*1	*k*2	*α*	*Rs*
BF	Lux Cellulose-1 MEOH/H_2O (90/10)	10	5.54	5.65	1.02	0.32
		15	5.08	5.17	1.02	0.27
		20	4.32	4.38	1.01	0.21
		25	4.05	4.05	1.00	0.00
		30	3.69	3.69	1.00	0.00
		35	3.34	3.34	1.00	0.00
		40	3.04	3.04	1.00	0.00
BF	Lux Cellulose-1 ACN/H_2O (70/30)	10	4.72	4.88	1.03	0.92
		15	4.61	4.76	1.03	0.56
		20	4.49	4.62	1.03	0.54
		25	4.42	4.54	1.03	0.52
		30	4.26	4.37	1.03	0.47
		35	4.09	4.19	1.02	0.46
		40	3.91	3.99	1.02	0.45
BF	Lux Cellulose-3 MEOH/H_2O (95/5)	10	1.77	6.01	3.39	8.02
		15	1.52	4.89	3.22	8.01
		20	1.60	4.95	3.09	8.32
		25	1.37	3.95	2.89	8.45
		30	1.38	3.82	2.78	8.60
		35	1.26	3.33	2.63	8.42
		40	1.14	2.84	2.49	8.25
BF	Lux Cellulose-3 ACN/H_2O (80/20)	10	0.56	1.84	3.29	7.82
		15	0.55	1.72	3.13	7.71
		20	0.52	1.58	3.05	7.63
		25	0.49	1.41	2.85	7.52
		30	0.47	1.26	2.67	7.30
		35	0.45	1.12	2.51	6.42
		40	0.41	0.98	2.37	6.10
LCT	Lux Cellulose-1 MEOH/H_2O (85/15)	10	3.96	4.79	1.21	1.63
		15	3.51	4.20	1.20	1.60
		20	2.91	3.43	1.18	1.47
		25	2.80	3.28	1.17	1.29
		30	2.50	2.90	1.16	1.25
		35	2.25	2.59	1.15	1.17
		40	2.06	2.35	1.14	1.09
LCT	Lux Cellulose-1 ACN/H_2O (70/30)	10	6.53	7.34	1.12	1.78
		15	6.23	6.95	1.12	1.75
		20	6.00	6.64	1.11	1.61
		25	5.65	6.24	1.11	1.59
		30	5.32	5.85	1.10	1.45
		35	4.99	5.47	1.09	1.35
		40	4.80	5.22	1.09	1.33
LCT	Lux Cellulose-3 MEOH/H_2O (95/5)	10	1.14	3.35	2.95	3.86
		15	1.01	2.76	2.75	4.64
		20	0.90	2.39	2.66	4.27
		25	0.81	2.00	2.48	4.02
		30	0.72	1.64	2.27	4.37
		35	0.64	1.38	2.16	4.64
		40	0.58	1.19	2.05	4.72

(Continues)

TABLE 2 (Continued)

Compound	Mobile Phase (vol/vol)	Temperature (°C)	*k*1	*k*2	*α*	*Rs*
LCT	Lux Cellulose-3 ACN/H_2O (60/40)	10	3.50	3.98	1.14	1.12
		15	3.45	3.94	1.14	1.53
		20	3.32	3.78	1.14	1.46
		25	3.23	3.68	1.14	1.55
		30	3.10	3.53	1.14	1.56
		35	2.96	3.36	1.14	1.58
		40	2.80	3.18	1.13	1.52

and longer elution time in enantiomer separation under reversed-phase HPLC. In accordance with this phenomenon, the retention factors (k) and resolution factor (R_s) values decreased with increasing ratio of methanol and acetonitrile in mobile phase. Previous study reported partial separation of LCT enantiomers on Chiralcel OD-R chiral column with maximum $R_s = 1.17$ under reversed-phase HPLC.[30] In the present study, the complete separation ($R_s > 1.5$) of BF and LCT enantiomers was achieved on both Lux Cellulose-1 and Lux Cellulose-3 column by using MEOH/H_2O and ACN/H_2O as mobile phase. The maximum R_s were 13.30 and 8.29 for BF and LCT, respectively. Thus, this study provided a baseline separation of BF and LCT enantiomers under reversed-phase HPLC.

3.2 | Influences of temperature

Column temperature is an important factor for chiral resolution and contributes to understanding the mechanism of chiral recognition. In this study, the influences of column temperature on BF and LCT enantiomer separation were carefully investigated from 10°C to 40°C with increment of 5°C on Lux Cellulose-1 and Lux Cellulose-3 columns (Figure 3). Table 2 summarized chromatographic conditions and separation results of temperature study. In BF enantiomer separation, the components of mobile phase were MEOH/H_2O (90/10) and ACN/H_2O (70/30) for Lux Cellulose-1, as well as MEOH/H_2O (95/5) and ACN/H_2O (80/20) for Lux Cellulose-3. As for LCT, MEOH/H_2O (85/15) and ACN/H_2O (70/30) were used as mobile phase on Lux Cellulose-1, as well as MEOH/H_2O (95/5) and ACN/H_2O (60/40) on Lux Cellulose-3 in consideration of eluted time and separation efficiency. The results indicated that temperature could significantly affect the interaction between 2 pyrethroid enantiomers and CSP. In general, lower temperature leads to longer eluted time, wider peak, and higher resolution. In our study, resolution factor (R_s) and retention factor (k_1, k_2) decreased with increasing temperature for BF and LCT enantiomers on Lux Cellulose-1. For instance, the R_s, k_1, and k_2 values of LCT enantiomers decreased from 1.78 to1.33, 6.53 to 4.80, and 7.34 to 5.22, respectively, on Lux Cellulose-1 column with ACN/H_2O ratio of 70/30. However, temperature sometimes has little effects on chiral resolution such as the separation of BF enantiomers on Lux Cellulose-3 with MEOH/H_2O ratio of 95/5 and the maximum R_s achieved at 30°C. Similar results were also obtained from chiral separation of LCT enantiomers on Lux Cellulose-3 (MEOH/H_2O [95/5]) and the maximum $R_s = 4.72$ obtained at 40°C.

3.3 | Thermodynamic parameters

To understand the thermodynamic driving forces involved in BF and LCT enantiomer separation, Van't Hoff equations were employed to calculate the standard enthalpy (ΔH) and entropy (ΔS) according to retention factors (k) and selectivity factor (α) obtained from different temperatures (Figure 4).

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln\varphi \quad (1)$$

$$\ln a = -\frac{\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R} \quad (2)$$

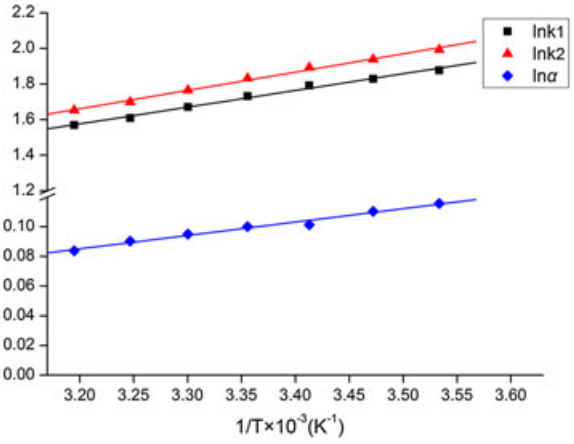

FIGURE 4 Van't Hoff plots of LCT on Lux Cellulose-1 with ACN/H_2O ratio of 70/30

TABLE 3 Van't Hoff equations and thermodynamic parameters of bifenthrin (BF) and lambda-cyhalothrin (LCT) enantiomers with Lux Cellulose-1 and Lux Cellulose-3 chiral columns

Compound	Column	Mobile Phase	$\ln k = -\Delta H/RT + \Delta S/R + \ln\varphi$	R^2	ΔH (KJ mol^{-1})	$\Delta S/R + \ln\varphi$	$\ln\alpha = -\Delta\Delta H/RT + \Delta\Delta S/R$	R^2	$\Delta\Delta H$ (KJ mol^{-1})	$\Delta\Delta S$ (J mol^{-1})
BF	Lux Cellulose-1	MEOH/H_2O (90/10)	$\ln k_1 = 1773.5/T - 4.5547$	0.994	−14.74	−4.55	—	—	—	—
			$\ln k_2 = 1842.6/T - 4.7795$	0.994	−15.32	−4.78	$\ln\alpha = 61.806/T - 0.1979$	0.979	−0.51	−1.65
BF	Lux Cellulose-1	ACN/H_2O (70/30)	$\ln k_1 = 542.82/T - 0.3536$	0.965	−4.51	−0.35	—	—	—	—
			$\ln k_2 = 571.28/T - 0.4219$	0.968	−4.75	−0.42	$\ln\alpha = 28.456/T - 0.0682$	0.995	−0.24	−0.57
BF	Lux Cellulose-3	MEOH/H_2O (95/5)	$\ln k_1 = 1165.4/T - 3.5636$	0.915	−9.69	−3.56	—	—	—	—
			$\ln k_2 = 2073.5/T - 5.5457$	0.965	−17.24	−5.55	$\ln\alpha = 908.15/T - 1.9821$	0.996	−7.55	−16.48
BF	Lux Cellulose-3	ACN/H_2O (80/20)	$\ln k_1 = 893.33/T - 3.7127$	0.976	−7.43	−3.71	—	—	—	—
			$\ln k_2 = 1878.5/T - 5.9856$	0.985	−15.62	−5.99	$\ln\alpha = 985.19/T - 2.2728$	0.984	−8.19	−18.90
LCT	Lux Cellulose-1	MEOH/H_2O (85/15)	$\ln k_1 = 1903.4/T - 5.3692$	0.987	−15.82	−5.37	—	—	—	—
			$\ln k_2 = 2073.7/T - 5.7819$	0.987	−17.24	−5.78	$\ln\alpha = 170.29/T - 0.4128$	0.994	−1.42	−3.43
LCT	Lux Cellulose-1	ACN/H_2O (70/30)	$\ln k_1 = 939.17/T - 1.4297$	0.992	−7.81	−1.43	—	—	—	—
			$\ln k_2 = 1029.3/T - 1.6331$	0.994	−8.56	−1.63	$\ln\alpha = 90.132/T - 0.2034$	0.982	−0.75	−1.69
LCT	Lux Cellulose-3	MEOH/H_2O (95/5)	$\ln k_1 = 1999.7/T - 6.9343$	0.999	−16.63	−6.93	—	—	—	—
			$\ln k_2 = 3087.2/T - 9.6891$	0.998	−25.67	−9.69	$\ln\alpha = 1087.5/T - 2.7548$	0.991	−9.04	−22.90
LCT	Lux Cellulose-3	ACN/H_2O (60/40)	$\ln k_1 = 657.77/T - 1.0513$	0.968	−5.47	−1.05	—	—	—	—
			$\ln k_2 = 673.26/T - 0.9742$	0.963	−5.60	−0.97	$\ln\alpha = 15.492/T + 0.0771$	0.623	−0.13	0.64

where R presents the gas constant (8.314 J/[mol K]), T is the absolute temperature, and φ is the phase ratio. ΔH and ΔS are the molecular enthalpy and entropy involved in enantiomer separation. $\Delta\Delta H$ represents the value of ΔH_2-ΔH_1, and $\Delta\Delta S$ is the value of ΔS_2-ΔS_1. Based on Equation 1, the values of $-\Delta H/R$ and $(\Delta S/R + \ln\varphi)$ could be calculated from the slop and intercept according to linear regression of ln$k$ versus $1/T$. Similarly, the values of $-\Delta\Delta H/R$ and $\Delta\Delta S/R$ could also be calculated based on linear regression of lnα versus $1/T$.

Table 3 summarized the thermodynamic parameters of BF and LCT enantiomers on Lux Cellulose-1 and Lux Cellulose-3 chiral columns by using MEOH/H_2O and ACN/H_2O as mobile phase. The ΔH values of BF and LCT enantiomers range from −4.51 to −17.24 kJ/mol and −5.47 to −25.67 kJ/mol, respectively. The negative values of ΔH clearly indicate that the transformation of BF and LCT enantiomers from mobile phase to CSP is mainly driven by enthalpy. The $\Delta\Delta H$ of BF and LCT enantiomers on 2 types chiral columns under specified

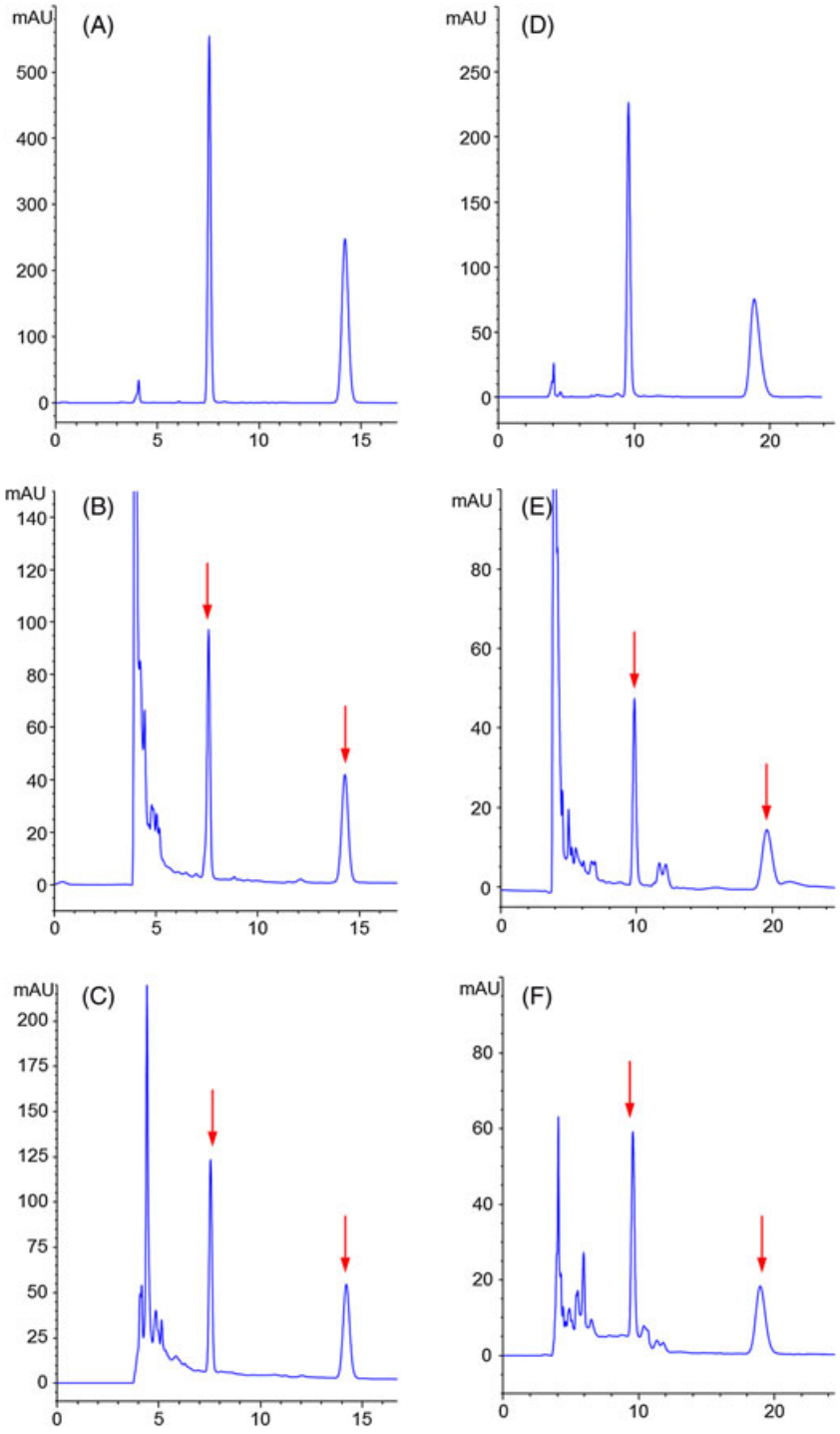

FIGURE 5 Representative chromatograms of bifenthrin (BF; Lux Cellulose-3, MEOH/H_2O = 95/5) and lambda-cyhalothrin (LCT; Lux Cellulose-3, MEOH/H_2O = 90/10) enantiomers extracted from soil and water. (A) BF standard solution; (B) BF extracted from water at 5 mg L^{-1} spiked level; (C) BF extracted from soil at 5 mg kg^{-1} spiked level; (D) LCT standard solution; (E) LCT extracted from water at 5 mg L^{-1} spiked level; (F) LCT extracted from soil at 5 mg kg^{-1} spiked level

mobile phase condition range from −0.24 to −8.19 kJ/mol and −0.13 to −9.04 kJ/, individually, which means the ΔH values of second eluted enantiomers are more negative than the first eluted enantiomer, implying that the CSPs have stronger interaction with the second eluted enantiomers than the first one. Moreover, the negative values of $\Delta\Delta H$ indicate that lower temperature leads to better separation of these 2 chiral pyrethroid enantiomers on Lux Cellulose-1 and Lux Cellulose-3 columns. However, the correlation coefficient (R^2) of linear regression of lnα versus $1/T$ is 0.623 on Lux Cellulose-3 with ACN/H_2O ratio of 60/40, which indicates that multiple interaction forces exist between LCT enantiomers and CSP.

3.4 | Bifenthrin and lambda-cyhalothrin enantiomer analysis in environmental samples

The developed chiral HPLC method was validated for the quantitative analysis of BF and LCT enantiomers in soil and water (Figure 5). The calibration curves obtained from the concentration range of 0.05 to 10 mg L^{-1} showed good linearity for the first eluted BF enantiomer ($y = 38.391x + 1.2195$, $R^2 = 0.9984$), second eluted BF enantiomer ($y = 42.428x + 0.6537$, $R^2 = 0.9999$), first eluted LCT enantiomer ($y = 34.188x + 2.7852$, $R^2 = 0.9998$), and second eluted LCT enantiomer ($y = 29.717x + 2.1693$, $R^2 = 1$), respectively. Average recoveries of BF enantiomers at 3 spiked levels were 91.42 to 99.74% from soil and 90.82 to 101.50% from water with the RSD ranging of 2.54 to 5.54% and 3.29 to 5.69%, respectively. The recoveries of LCT enantiomers were 90.83 to 100.30% from soil and 92.64 to 98.48% from water with RSD less than 7% (Table 4). The LODs for BF and LCT enantiomers were 0.01 and 0.015 mg L^{-1}, respectively, and the corresponding LOQ is 0.05 mg L^{-1} for both BF and LCT based on the lowest concentration in calibration curve with acceptable RSD < 20%. Such results indicated that the methods were robust and reliable for residual analysis of BF and LCT enantiomers in environmental samples.

4 | CONCLUSION

In the present study, type I (BF) and type II (LCT) pyrethroid enantiomers were separated on Lux Cellulose-1, Lux Cellulose-3, and Chiralpak IC chiral columns by reversed-phase high-performance liquid chromatography. Bifenthrin gets a baseline separation on Lux Cellulose-3 and partial separation on Lux Cellulose-1 with maximum $R_s = 13.30$ and $R_s = 0.54$, respectively, while LCT enantiomers could be completely separated on Lux Cellulose-1 and Lux Cellulose-3 with maximum $R_s = 2.03$ and $Rs = 8.29$, individually. Chiralpak IC provides no separation ability for both BF and LCT. ACN/H_2O exhibits better separation efficiency than MEOH/H_2O on Lux Cellulose-1 and Lux Cellulose-3 chiral columns for BF, whereas MEOH/H_2O is much better than ACN/H_2O on Lux Cellulose-3, and these 2 mobile phases exhibit similar separation potency on Lux Cellulose-1 for LCT. Retention factor (k) and selectivity factor (α) decreased with the column temperature increasing from 10°C to 40°C for both BF and LCT. Furthermore, the quantitative analysis methods for BF and LCT enantiomers in soil

TABLE 4 Recovery and precision of bifenthrin (BF) and lambda-cyhalothrin (LCT) enantiomers from soil and water (n = 3)

Sample	Spiked Levels (mg kg^{-1} or mg L^{-1})	E1 (First Eluted Enantiomer)		E2 (Second Eluted Enantiomer)	
		Recovery (%)	RSD	Recovery (%)	RSD
BF					
Soil	0.02	98.67 ± 4.25	4.31	99.74 ± 5.01	5.03
	0.5	95.63 ± 5.30	5.54	91.42 ± 2.33	2.54
	5	96.15 ± 3.51	3.65	97.42 ± 3.78	3.88
Water	0.02	93.35 ± 5.08	5.44	95.69 ± 5.12	5.35
	0.5	101.50 ± 3.75	3.70	93.31 ± 4.43	4.74
	5	90.82 ± 5.16	5.69	94.45 ± 3.11	3.29
LCT					
Soil	0.02	100.30 ± 4.59	4.58	98.69 ± 5.34	5.42
	0.5	97.25 ± 4.15	4.27	96.56 ± 4.76	4.93
	5	99.76 ± 3.70	3.71	90.83 ± 3.99	4.40
Water	0.02	97.35 ± 6.50	6.68	93.06 ± 4.43	4.76
	0.5	93.67 ± 4.81	5.13	98.48 ± 4.73	4.80
	5	96.80 ± 4.52	4.67	92.64 ± 4.63	5.00

and water were also established. Such results provide a new approach for pyrethroid separation under reversed-phase condition and contribute to risk assessment of pyrethroids at enantiomer level.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interest.

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