Enantiomeric Resolution of Five Chiral Pesticides on a Chiralpak IB-H Column by SFC

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Abstract

The enantiomeric separations of five chiral pesticides, diclofop-methyl, 1; benalaxy, 2; acetofenate, 3; myclobutanil, 4; and difenoconazole, 5, were conducted on a Chiralpak IB-H column by a packed-column supercritical fluid chromatography (p-SFC). All compounds, except difenoconazole and myclobutanil, were well resolved within 10 min. As the mobile phase polarity decreased through changing the percentage and the type of alcohol modifiers in the supercritical carbon dioxide (CO_2), the retention time, the separation factors, and the resolution increased. However, based on the retention time and the resolution, the optimized separations were obtained with the mobile phase containing 10% 2-propanol for diclofop-methyl 1; benalaxy, 2; myclobutanil, 4; difenoconazole, 5; and containing 3% 2-propanol for acetofenate, 3. The optimized separation temperature was at 35°C under the supercritical fluid condition. The π–π interactions and the hydrogen bonding interactions between Chiralpak IB-H CSP and the analytes might be the main chiral discriminations on enantioseparation of these five pesticides.

Introduction

The chirality of pesticides has attracted great attention in recent years. Chiral compounds account for more than one quarter of the commercialized agrochemical compounds in the 1990s (1), and to date, this ratio has increased to about 30% (2). These compounds mainly include synthetic pyrethroids insecticides, triazole-related fungicides, cyclohexanedione imidazolinones, and phenoxypropanoic-acid herbicides. As known, two enantiomers of chiral compounds often show great differences in terms of their bioavailablity, distriubution, and metabolic and excretion behavior. For example, the insecticidal activity of metalaxyl or valerate, and the herbicidal activity of metolachlor, haloxyfop, or napropamide is mainly from one of their two enantiomers. The insecticidal activity of permethrin or fenvalerate, with their two chiral centres, is mainly from one of their four enantiomers. And the insecticidal activity of cypermethrin, dehamethrin, or allethrins, with their three chiral centres, is from one of their eight enantiomers. The (+)-enantiomer of the synthetic pyrethroids, bifenthrin, or permethrin, is 17–38-fold acute toxicity to the freshwater invertebrates *Ceriodaphnia dubia* and *Daphnia magna* as the (–)-enantiomer (3). For the herbicides, S-metolachlor was more toxic to *Chlorella pyrenoidosa* than rac-metolachlor, and the catalase activity of *Chlorella pyrenoidosa* treated by S-metolachlor was higher than that exposed to rac-metolachlor (4). Meanwhile, S-metolachlor degraded faster in soil than rac-metolachlor (5–7). Consequently, commercialization with the pure enantiomer of chiral pesticides is necessary and meaningful for saving resources.

One of the challenges in studying the enantioselectivity of chiral pesticides is the separation of enantiopure isomers. Many separation techniques have been used to obtain pure enantiomers, and undoubtedly the most used one is the chromatographic method. Today's enantiomeric separations are mainly carried out using high-performance liquid chromatography (HPLC) (8–10). Owing to the high efficiency and short retention time, packed-column supercritical fluid chromatography (p-SFC) has shown better separation results than HPLC (11–14). The higher diffusivity of supercritical CO_2 as the mobile phase, compared with liquids, results in better separation efficiencies, and the lower viscosity offers lower pressure drops with higher flow rates (15). Nowadays, chromatographers have an increased interest for enantioseparation by SFC using an all LC chiral stationary phase (CSPs), except for the chiral crown ester CSPs and the protein-based CSPs (16). The polysaccharide CSPs are one of the most applied CSPs for enantioseparation, both in analytical and preparative SFC and by virtue of their broad enantioselective resolution (17). However, the immobilized polysaccharides CSPs have been rarely applied in the SFC separation of pesticides. Recently, the efforts in separating the three new neonicotinoid insecticides on a Chiralpak IB by SFC was in vain (18). A successful separation of pesticides on a Chiralpak IB by SFC has not been reported yet.

Pesticides containing one or two chiral centres, including diclofop-methyl, 1; benalaxy, 2; acetofenate, 3; myclobutanil, 4; and difenoconazole, 5, have been successfully separated by HPLC on different polysaccharide-based and pirkle-based CSPs (19–27). This was achieved through a capillary LC, using teicoplanin as the chiral selector (28) and by CD-MEKC (micellar electrokinetic chromatography), containing 40 mM 2-hydroxypropyl-γ-CD + 50 mM sodium dodecyl sulfate in a 25 mM phos-

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phate buffer (pH 3.0) (29). The difference between Chiralcel OD-H and Chiralpak IB-H is the combination method that whether the chiral selector is physically coated (for the former) or chemically immobilized (for the latter) onto the silica gel; thereby, the separation results of these pesticides on a Chiralcel OD-H by HPLC could be the reference. For diclofop-methyl and benalaxyl, the resolutions conducted on coated cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) CSP with *n*-hexane–2-propanol (98:2) by HPLC were 5.32 and 4.39, respectively (20). Acetofenate was well separated by HPLC on Chiralcel OD using a 100% *n*-hexane as the mobile phase (Rs 4.18), according to the research by Xu et al. (22). An excellent baseline separation of the myclobutanil enantiomers was obtained using 1.20 mol/L ethanol in *n*-hexane (*n*-hexane–ethanol, 93:7, v/v), with α 2.60 and Rs 15.53 on CDMPC CSP (300 × 4.0 mm i.d., 20 µm) in Pan's report (19). In this paper, these five pesticides were separated by SFC on an analytical Chiralpack IB-H column for the first time, and the effects of the alcohol modifiers and the separation temperatures on the retention factor, enantioselectivity, and resolution were evaluated. In addition, chiral recognition between these pesticides and bonded Chiralpak IB-H in SFC was discussed.

Materials and Methods

Chemicals and reagents

The racemic compounds used in this study (Figure 1), diclofop-methyl, 1; benalaxy, 2; acetofenate, 3; myclobutanil, 4; and difenoconazole, 5 were kindly donated by Yifan Chemical Co., Ltd (Wenzhou, China). Stock solutions of all analytes were prepared by dissolution in ethanol at a concentration of approximately 100 mg/L. The organic solvents, of HPLC grade, were obtained from Tjshield (Tianjin, China). CO_2, with a purity of 99.9%, was purchased from Jingong Specialty Gas Co. (Hangzhou, China).

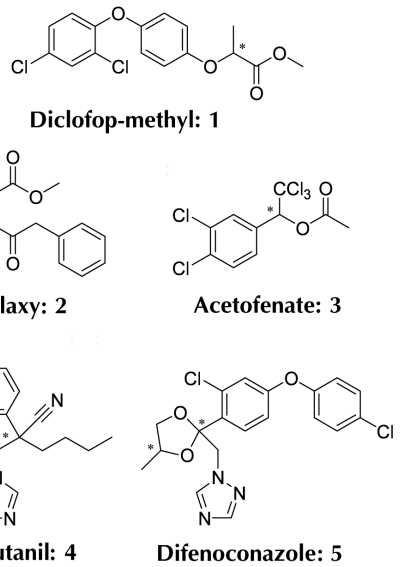

Figure 1. Chemical structures of the pesticides; *denotes chiral centre.

Apparatus and methods

The SFC experiments were performed on a Thar SD-ASFC-2 system (Thar Technologies, Pittsburgh, PA) equipped with a UV–vis-151 detector. The system was controlled by Superchrom software. The enantioseparation was performed on a Chiralpak IB-H [cellulose tris(3,5-dimethylphenylcarabamate), and immobilized onto silica gel, 250 × 4.6 mm i.d., 5 µm]. The chromatography was conducted using CO_2 composed with different types and percentages of alcohol modifiers as the mobile phase. For the separation experiments, the total flow rate of the mobile phase, the outlet backpressure, the injection volume, and the UV detection wavelength were fixed at 2.0 mL/min, 150 bar, 20 µL, and 220 nm, respectively. The oven temperature was set at 35°C, unless noted otherwise, to determine the effect of temperature on enantiomeric separation.

The chromatographic parameters, including the retention factor (k), the separation factor (α), and the resolution (Rs) were selected to evaluate the separation of the compounds. All the chromatographic results were repeated three times.

Results and Discussion

The enantioseparation of the five pesticides was conducted on a Chiralpak IB-H by HPLC using *n*-hexane with 2-propanol as the mobile phase. However, only diclofop-methyl and benalaxyl were well separated with *n*-hexane–2-propanol (85:15) with α 1.99, Rs 9.86, and α 1.47, Rs 5.50, respectively. Acetofenate was separated with α 1.21, Rs 2.93, using 100% n-hexane as the mobile phase. For myclobutanil, there was no peek eluted with 85/15 *n*-hexane/2-propanol in 60 min. With the increase of n-hexane percents, the retention time on the Chiralpak IB-H for these pesticides increased dramatically. Specially, the separation is highly specific in SFC analysis. All the five pesticides were baseline separated on the Chiralpak IB-H in SFC with the optimization of the chromatographic conditions except difenoconazole. All the chromatographic results were highly reproduced.

Effects of alcohol modifiers

The percentages and the types of the polar modifier for the mobile phase can influence the enantiomers' elution time and resolution greatly, and even can change the elution order (30–31). Methanol, ethanol, or 2-propanol with different percentages was chosen as the polar modifiers of the SFC mobile phase to search for the best enantiomeric selectivity for the five pesticides.

As shown in Table I, at 35°C, 150 bar, high resolutions of all compounds were obtained by using all of the alcohol modifiers on Chiralpak IB-H column in SFC except difenoconazole, which contains two chiral centres as four enantiomers. As the mobile phase polarity decreased (i.e., the alcohol modifier changed from methanol, ethanol to 2-propanol, and/or the alcohol concentration changed from 15%, 10%, 5%, to 3%), the retention time increased, as well as the separation factors and the resolution. However, in the case of difenoconazole, ethanol gave a little better separation than 2-propanol, though neither of them could provide efficient separation. For myclobutanil, 15% 2-propanol

as the mobile phase modifier gave a higher resolution than 10% 2-propanol (Rs 19.84 versus 18.69). Generally, the best resolution of these pesticides was mainly obtained with 3% 2-propanol in CO_2, especially for acetofenate, which were baseline separated only with 5% and 3% 2-propanol. For diclofop-methyl and benalaxyl, excellent baseline separation was achieved with 3% 2-propanol by SFC, with resolutions 8.25 and 5.00, respectively. The retention of acetofenate was so weak that the mobile phase, which consisted of 100% CO_2, eluted its two enantiomers in 5 min (data not shown). However, myclobutanil was strongly retained on the relevant CSP (t'2 133.2 min with 3% 2-propanol), and with 20% 2-propanol in CO_2, α 2.88, and Rs 15.27 were obtained approximately in 10 min (data not shown). Hence, considering both the retention time and the resolution of the pesticides, the best separation results were obtained with 10% 2-propanol in CO_2, except for acetofenate with 3% 2-propanol. Additionally, the resolution of these pesticides in the ascending order was difenoconazole < acetofenate < benalaxyl < diclofop-methyl < myclobutanil. Typical separation chromatograms of these pesticides are shown in Figure 2.

Table I. Chromatographic Separation Results for Five Pesticides on Chiralpak IB-H by SFC

		Methanol (%)				Ethanol (%)				2-Propanol (%)			
Compound		15	10	5	3	15	10	5	3	15	10	5	3
1	k_1	0.93	1.29	2.34	2.34	0.73	0.91	2.23	3.64	0.91	1.51	3.19	5.47
	k_2	1.06	1.49	2.73	2.68	0.90	1.16	2.84	4.77	1.25	2.07	4.40	7.76
	α	1.13	1.15	1.17	1.15	1.22	1.27	1.27	1.31	1.37	1.37	1.38	1.42
	R_s	1.86	2.47	3.08	3.15	2.72	3.26	4.27	5.90	4.88	5.58	7.22	8.25
2	k_1	0.42	0.75	1.75	1.71	0.49	0.91	2.15	3.77	0.83	1.52	3.55	6.47
	k_2	0.53	0.92	2.15	2.14	0.64	1.15	2.73	4.95	1.06	1.93	4.63	8.55
	α	1.26	1.23	1.23	1.25	1.32	1.27	1.37	1.32	1.27	1.27	1.30	1.32
	R_s	1.84	2.09	2.92	3.65	2.45	3.00	3.90	5.38	2.72	3.47	4.57	5.00
3	k_1	0.27	0.30	0.51	0.52	0.13	0.21	0.45	0.64	0.18	0.30	0.56	0.82
	k_2	0.27	0.30	0.55	0.56	0.13	0.23	0.49	0.72	0.18	0.34	0.63	0.95
	α	1	1	1.08	1.07	1	1.14	1.10	1.12	1	1.13	1.13	1.15
	R_s	–	–	0.91	0.88	–	0.83	1.09	1.50	–	1.02	1.54	2.12
4	k_1	1.32	2.52	6.13	12.3	1.73	3.27	8.64	18.1	3.58	5.80	16.3	31.6
	k_2	2.75	5.31	13.7	29.0	4.09	7.80	21.0	46.6	10.5	15.0	43.7	87.8
	α	2.08	2.11	2.24	2.36	2.36	2.38	2.43	2.58	2.91	2.60	2.68	2.78
	R_s	12.1	14.4	17.5	19.2	15.4	18.5	19.4	21.8	19.8	18.7	21.4	21.7
5	k_1	2.09	3.75	9.04	17.0	1.55	3.91	5.40	19.6	3.11	5.96	16.3	33.0
	k_2	2.63	4.94	11.8	21.7	2.08	4.66	6.20	23.1	3.63	6.97	19.0	38.0
	k_3	–	–	–	22.6	2.75	4.83	6.44	24.8	3.86	7.48	20.9	41.8
	k_4	–	–	–	–	–	5.26	6.82	–	4.07	7.78	–	–
	α_1	1.26	1.32	1.31	1.27	1.18	1.19	1.15	1.18	1.17	1.17	1.17	1.15
	α_2	–	–	–	1.04	1.12	1.04	1.04	1.07	1.06	1.07	1.10	1.10
	α_3	–	–	–	–	–	1.09	1.06	–	1.05	1.04	–	–
	R_{s12}	3.19	3.91	4.30	6.14	2.80	3.81	3.43	3.80	2.82	3.14	3.45	2.91
	R_{s23}	–	–	–	0.86	1.45	0.77	0.92	1.38	1.14	1.48	1.85	1.83
	R_{s34}	–	–	–	–	–	1.73	1.26	–	0.91	0.81	–	–

Table II. Effect of Temperature on the Separation of Five Pesticides on Chiralpak IB-H by SFC

Compound		k_1	k_2	α_1	R_{s12}	Compound		k_1	k_2	α_1	R_{s12}
1	25°C	1.18	1.63	1.38	5.25	2	25°C	1.26	1.55	1.23	2.47
	30°C	1.18	1.62	1.37	5.09		30°C	1.27	1.56	1.22	2.54
	35°C	1.51	2.07	1.37	5.58		35°C	1.52	1.93	1.27	3.47
	40°C	1.30	1.71	1.32	4.96		40°C	1.38	1.66	1.20	2.43
3	25°C	0.59	0.71	1.21	2.22	4	25°C	5.87	15.8	2.68	17.7
	30°C	0.63	0.75	1.19	2.11		30°C	5.81	15.4	2.65	17.3
	35°C	0.82	0.95	1.15	2.12		35°C	5.80	15.0	2.60	18.6
	40°C	0.73	0.87	1.19	2.38		40°C	6.28	15.5	2.47	19.0

Compound		k_1	k_2	k_3	k_4	α_1	α2	α3	R_{s12}	R_{s23}	R_{s34}
5	25°C	5.05	5.58	6.13	–	1.10	1.10	–	1.40	1.31	–
	30°C	5.17	5.73	6.24	–	1.11	1.09	–	1.38	1.19	–
	35°C	5.96	6.97	7.48	7.78	1.17	1.07	1.04	3.14	1.48	0.81
	40°C	5.83	6.29	6.61	6.88	1.08	1.05	1.04	1.48	1.01	0.71

Effects of separation temperatures

As mentioned, considering both the retention and the resolution of the pesticides, the best separation results were obtained with 10% 2-propanol in CO_2, except acetofenate with 3% 2-propanol. Hence, the effect of temperature on chiral separation of five pesticides was conducted in the range of 25°C~40°C at intervals of 5°C on the Chiralpak IB-H, with 10% 2-propanol as the mobile phase modifier (except acetofenate with 3% 2-propanol) at the back pressure 150 bar. The values of k, α, and Rs at various temperatures are shown in Table II.

The effect of the temperature on chiral separation by SFC is very complicated. Keeping the pressure constant, the variation of the temperature can affect the retention from two aspects (32). On one hand, the adsorption of both enantiomers was weakened by the increase of temperature, which caused the decrease of retention. On the other hand, the density of the near critical mobile phase decreased dramatically with the increase of temperature, especially beyond the critical temperature, which resulted in the increase of retention. Hence, the retention of the analytes may be controlled by the dominating one of these two aspects, and enantioselectivity could be improved by accommodating the appropriate column temperature. The separation temperature influences chiral retention both on the dynamics, which affects the viscosity and diffusion coefficient of the solute in the mobile phase, and the thermodynamics with the van't Hoff equations:

$$\ln k = (-\Delta H°/RT) + (\Delta S°/R) + \ln ø \qquad \text{Eq. 1}$$

$$\ln \alpha = (-\Delta\Delta H°/RT) + (\Delta\Delta S°/R) \qquad \text{Eq. 2}$$

As shown in Table II, for most compounds except myclobutanil, with an increased temperature, the k, α, and Rs values increased almost to the maximum values at 35°C and then decreased. It may be that beyond 35°C, the decreased density of the mobile phase was the main influential factor, and above 35°C, the adsorption effect preponderated. Retention increases beyond the critical temperature is a typical behavior of near critical mobile phases (33). All the phenomena might be

explained that 35°C is the point nearest to the critical temperature of CO_2 (31.1°C) and is higher than this critical temperature. However, for myclobutanil, the largest retention time was obtained at 40°C. It might be explained that the decrease of the density causes a reduction of the solvating power of the mobile phase, so the retention of the analytes increased. The maximum k, α, and Rs values for myclobutanil were obtained at 40°C, 25°C, and 40°C, respectively, and the highest Rs value for acetofenate was 2.38 at 40°C. The relationship of lnk and lnα to 1/T for the five pesticides are nonlinear, as shown in Figure 3. Consequently, the supercritical fluid condition showed some superiority to the sub-supercritical fluid condition for the chiral separation here.

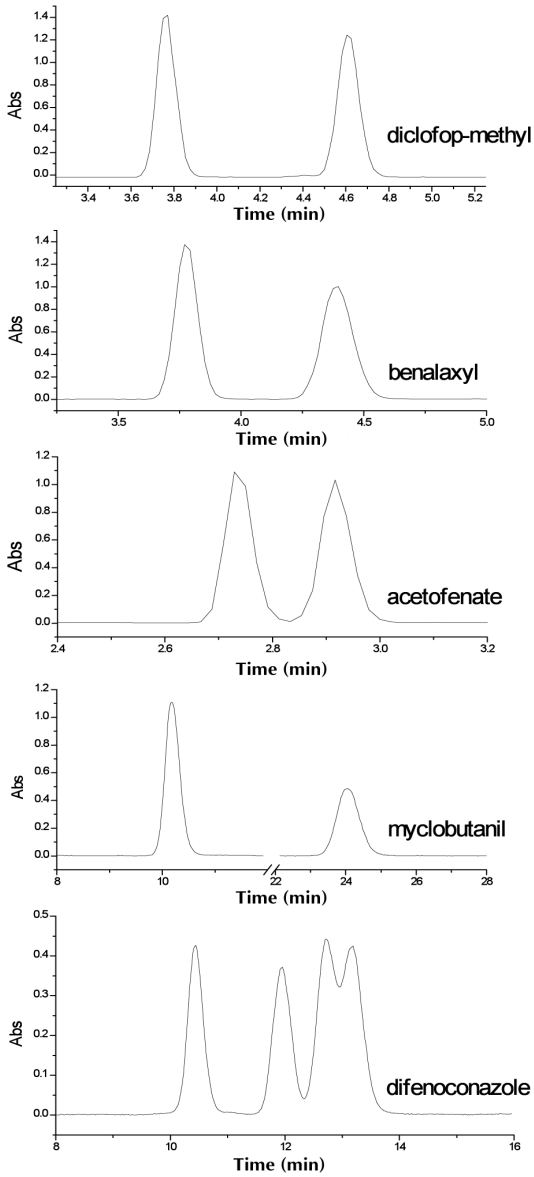

Figure 2. Optimal chromatograms of the five pesticides by SFC. Chromatographic conditions: Chiralpak IB-H column with 10% 2-propanol in CO2 for diclofop-methyl, 1; benalaxy, 2; acetofenate, 3; myclobutanil, 4; and difenoconazole, 5; and 3% 2-propanol in CO_2 acetofenate, 3; flow rate: 2.0 mL/min; column temperature: 35°C; backpressure: 150 bar.

Chiral recognition

Chiral recognition occurs while the interactions between the CSP and the two enantiomers are different. To the CSP Chiralpak IB-H, the D-(+)-glucose groups of the cellulose link as chiral cavities, bringing the steric interactions, and the derivative groups 3,5-dimethylphenyl carbamates provide phenyl groups, which are linked with two $–CH_3$ groups as the electron-donating groups, carbonyl groups (C=O), and –NH groups. To the racemates, all of the

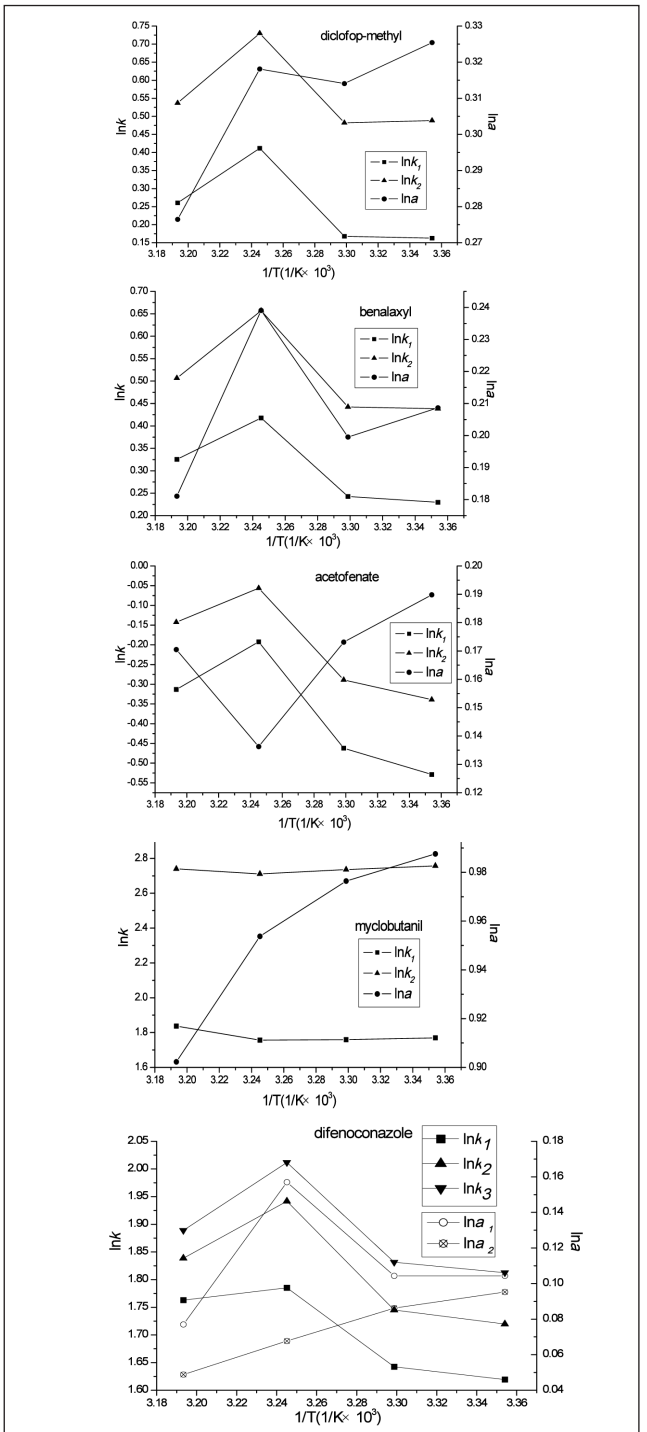

Figure 3. The van't Hoff plots of k and α for the enantiomers of five pesticides by SFC. Chromatographic conditions: Chiralpak IB-H column; 10% 2-propanol in CO_2, except for acetofenate with 3% 2-propanol; at the flow rate of 2.0 mL/min, backpressure of 150 bar.

five pesticides contain a phenyl ring directly attached or indirectly attached to the chiral C centre (one of the difenoconazole chiral centres) via an electronegative atom (nitrogen or oxygen). Hence, all the pesticides act as the steric interactions by the phenyl ring and the chiral cavities, as well as the π–π interactions between the phenyl ring of compounds and the phenyl acylamide groups of the CSP. The chiral discrimination mechanisms may include: the π–π interactions created between the phenyl rings of the compounds and phenyl rings of CSP for all the tested pesticides, and the electron-withdrawing groups –Cl on the para-position of phenyl ring of compounds show some favour to the π–π interactions (diclofop-methyl, acetofenate, and myclobutanil); the dipole-dipole interactions interact between a carbonyl group (C=O) of the compounds and a C=O group of CSP for diclofop-methyl, benalaxy, and acetofenate (weak); the hydrogen bonding interactions created between a C=O group of the analytes and a –NH group of CSP for diclofop-methyl, benalaxy and acetofenate (weak); the hydrogen bonding interactions created between an ether group of the compounds and a –NH group of CSP for diclofop-methyl, acetofenate, and difenoconazole, and these hydrogen bonding interactions may be reduced or hindered by the –Cl groups on ortho- or meta-position of phenyl ring next to the chiral centre of the analytes. Additionally, the poor retention of acetofenate may be due to the weakening of the π–π interactions which were caused by the less phenyl ring and the decrease of the electron density at the phenyl ring because of the substituents –Cl on the ortho-position of the phenyl ring. Special for myclobutanil, the electron-withdrawing –CN groups may enhance the chiral inductive effects greatly. Consequently, the π–π interactions and the hydrogen bonding interactions between CSP and the analytes could be the main chiral discriminations on enantioseparation of these five pesticides.

Conclusions

Results of the present study demonstrated that four of five chiral pesticides used in this study could be successfully separated on the Chiralpack IB column by SFC, except for difenoconazole with partial separation. The choice of the organic modifier depends on the compound to be separated, while supercritical fluid shows some superiority over sub-supercritical fluid for chiral separation. Additionally, it was deduced that the π–π interactions and the hydrogen bonding interactions between CSP and compounds were beneficial to the enantioseparation of these five pesticides.

Acknowledgements

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References

1. A. Williams, Opportunities for chiral agrochemicals. *Pestic. Sci.* **46:** 3–9 (1996).
2. Y.B. Zhang, New progress of pesticides in the world. Chemical Industry Press, Beijing: 114 (2007).
3. W.P. Liu, et al., Enantioselectivity in environmental safety of current chiral insecticides. *Proc. Natl. Acad. Sci. U.S.A.* **102:** 701–706 (2005).
4. H.J. Liu and M.Y. Xiong. Comparative toxicity of racemic metolachlor and S-metolachlor to Chlorella pyrenoidosa. *Aquat. Toxicol.* **93:** 100–106 (2009).
5. Y. Ma, et al., Enantioselective degradation of rac-metolachlor and S-metolachlor in soil. *Pedosphere* **16:** 489–494 (2006).
6. W. Liu and Y. Ma. Enantioselective degradations of (RAC)-metolachlor and (S)-metolachlor in Chinese soils. *Abstr. Am. Chem. Soc.* **229:** U80 (2005).
7. P.B. Kurt-Karakus, et al., Comparison of concentrations and stereoisomer ratios of mecoprop, dichlorprop and metolachlor in Ontario streams, 2006–2007 vs. 2003–2004. *Environ. Pollut.* **158:** 1842–1849 (2010).
8. E. Yashima, Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation. *J. Chromatogr. A* **906:** 105–125 (2001).
9. Y. Okamoto; Yashima, E., Polysaccharide derivatives for chromatographic separation of enantiomers. *Angew. Chem., Int. Ed.* **37:** 1020–43 (1998).
10. C. Perrin, et al., Screening approach for chiral separation of pharmaceuticals Part I. Normal-phase liquid chromatography. *J. Chromatogr. A* **947:** 69–83 (2002).
11. W.H. Pirkle, et al., Liquid and subcritical CO2 separations of enantiomers on a broadly applicable polysiloxane chiral stationary phase. *J. Chromatogr. A* **753:** 109–119 (1996).
12. M. Garzotti and M. Hamdan, Supercritical fluid chromatography coupled to electrospray mass spectrometry: a powerful tool for the analysis of chiral mixtures. *J. Chromatogr. B: Anal Technol. Biomed Life Sci.* **770:** 53–61 (2002).
13. D. Mangelings and Y.V. Heyden, Chiral separations in sub- and supercritical fluid chromatography. *J. Sep. Sci.* **31:** 1252–1273 (2008).
14. L.T. Taylor, Supercritical fluid chromatography for the 21st century. *J. Supercrit. Fluids* **47:** 566–573 (2009).
15. A. Rajendran, et al., Enantioseparation of 1-phenyl-1-propanol on Chiralcel OD by supercritical fluid chromatography -I. Linear isotherm. *J. Chromatogr. A* **1076:** 183–188 (2005).
16. R. Duval, et al., Enantioseparation of aminoglutethimide and thalidomide by high performance liquid chromatography or supercritical fluid chromatography on mono-2 and mono-6-O-pentenyl-β-cyclodextrin-based chiral stationary phases. *Biomed. Chromatogr.* **15:** 202–206 (2001).
17. C. White, Integration of supercritical fluid chromatography into drug discovery as a routine support tool-Part I. Fast chiral screening and purification. *J. Chromatogr A* **1074:** 163–173 (2005).
18. C. Zhang, et al., Chiral separation of Neonicotinoid insecticides by polysaccharide-type stationary phases using high-performance liquid chromatography and supercritical fluid chromatography. *Chirality* **23:** 215–221 (2011).
19. C.X. Pan, et al., Comparative enantioseparation of seven triazole fungicides on (S,S)-Whelk O1 and four different cellulose derivative columns. *J. Sep. Sci.* **29:** 2004–2011 (2006).
20. P. Wang, et al., Enantiomeric resolution of chiral pesticides by high-performance liquid chromatography. *J. Agric. Food Chem.* **54:** 1577–1583 (2006).
21. P. Wang, et al., The chiral resolution of pesticides on amylose-tris(3,5-dimethylphenylcarbamate) CSP by HPLC and the enantiomeric identification by circular dichroism. *Chirality* **20:** 40–46 (2008).
22. C. Xu, et al., Enantioselectivity in zebrafish embryo toxicity of the insecticide acetofenate. *Chem. Res. Toxicol.* **21:** 1050–55 (2008).
23. Y. Zhou, et al., Enantiomer separation of triazole fungicides by high-performance liquid chromatography. *Chirality* **21:** 421–427 (2009).
24. J.L. Diao, et al., Environmental behavior of the chiral aryloxyphenoxypropionate herbicide diclofop-methyl and diclofop: enantiomerization and enantioselective degradation in soil. *Environ. Sci. Technol.* **44:** 2042–2047 (2010).
25. Q. Tian, et al., Direct Enantiomeric separation of chiral pesticides by LC on amylose tris(3,5-dimethylphenylcarbamate) stationary phase under reversed phase conditions. *Chromatographia* **71:** 855–865 (2010).
26. P. Wang, et al., Enantiomeric separation of chiral pesticides by high-performance liquid chromatography on an amylose tris-(S)-1-phenylethylcarbamate chiral stationary phase. *J. Sep. Sci.* **29:** 265–271 (2006).
27. P. Wang, et al., The direct chiral separations of fungicide enantiomers on amylopectin based chiral stationary phase by HPLC. *Chirality* **19:** 114–119 (2007).
28. N. Rosales-Conrado, et al., Multivariate optimization approach for chiral resolution of chlorophenoxy acid herbicides using teicoplanin as chiral selector in capillary LC. *Chromatographia* **67:** 527–533 (2008).
29. W.A.W. Ibrahim, et al., Stacking and sweeping in cyclodextrin-modified MEKC for chiral separation of hexaconazole, penconazole and myclobutanil. *Chromatographia* **71:** 305–309 (2010).
30. J. Haginaka, et al., Chiral separation of propranolol and its ester derivatives on an ovomucoid-bonded silica: Influence of pH, ionic strength and organic modifier on retention, enantioselectivity and enantiomeric elution order. *Chromatographia* **29:** 587–92 (1990).
31. J. Haginaka, et al., Retention, enantioselectivity and enantiomeric elution order of propranolol and its ester derivatives on an Alpha1-acid glycoprotein-bonded column. *Chromatographia* **33:** 127–32 (1992).
32. B.G. Su, et al., Enantioseparation of paroxetine intermediate on an amylose-derived chiral stationary phase by supercritical fluid chromatography. *J. Chromatogr. A* **1216:** 5140–46 (2009).
33. Y.Q. Zhao, et al., Chiral separation of selected proline derivatives using a polysaccharide-type stationary phase by supercritical fluid chromatography and comparison with high-performance liquid chromatography. *J Chromatogr A* **1189:** 245–53 (2008).

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