CHIRALITY 23:215–221 (2011)

Chiral Separation of Neonicotinoid Insecticides by Polysaccharide-Type Stationary Phases Using High-Performance Liquid Chromatography and Supercritical Fluid Chromatography

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ABSTRACT The enantiomeric separations of three neonicotinoid insecticides (identified as compounds **1**, **2**, and **3**) were performed on three polysaccharide-type chiral columns, that is, Chiralcel OD-H, Chiralpak AD-H, and Chiralpak IB, by high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). Effects of the modifier percentage and column temperature on chiral recognitions of chiral stationary phases were also studied. Both **1** and **2** could be resolved on all three columns selected, with the highest R_s values obtained on Chiralpak AD-H and Chiralcel OD-H, respectively. However, satisfactory separation of the four stereoisomers of **3** was only achieved on Chiralcel OD-H. Considering the effects of ethanol on the values of k, α, and R_s, we concluded that hydrogen bonding, π–π, and/or dipole–dipole interactions might be all responsible for the chiral separation. In comparison to HPLC, a shorter run time was achieved for **1** and **2** by SFC. However, **3** could not be stereoselectively resolved using SFC. On the basis of the calculated thermodynamic parameters, we found that the separation processes of enantiomers of **1** and **2** were entropy controlled and enthalpy controlled, respectively. *Chirality 23:215–221, 2011.*

KEY WORDS: enantiomeric separation; neonicotinoid insecticides; polar modifier; column temperature

INTRODUCTION

Chirality is the rule rather than the exception in the universe, as living organisms are anisotropic on either microscales or macroscales. This molecular chirality in life science has been well recognized and applied in the pharmaceutical industry, where 50 of the top 100 most widely sold drugs are marketed as single enantiomers to avoid adverse side effects.[1,2] In the last two decades, great attention has also been paid to the stereoselective properties of currently used pesticides, of which up to 25% were chiral in the 1990s,[3] and this ratio has now increased to about 40%, as compounds with more complex structures are being registered for use.[4] Numerous examples have demonstrated that many biological activities such as insecticidal activity, aquatic toxicity, development toxicity, endocrine disruption, immunotoxicity, and microbial degradation of chiral pesticides are enantioselective.[5–10] As a result, it is very important to assess the pesticidal activity and environmental safety of chiral pesticides in their enantiopure forms.[11]

One of the biggest challenges in the study of the enantioselective profiles of chiral pesticides is the separation and analysis of pure enantiomers. With various inherent advantages such as rapid analysis, reproducibility, and flexibility, high-performance liquid chromatography (HPLC) with different chiral stationary phases (CSPs) has become one of the most widely used methods for today's enantiomeric separations.[12,13] Nevertheless, in the last few years, supercritical fluid chromatography (SFC) has emerged as a powerful alternative. The physicochemical properties of the supercritical fluids allow to obtain separations with short analysis time, high separation efficiency, low solvent consumption, and environmental friendliness. A wide range of CSPs have been used in SFC.[14–17]

Neonicotinoids are a class of insecticides that act on insect nicotinic acetylcholine receptors. They have outstanding effectiveness toward the target organisms but exhibit relatively low toxicity to vertebrates. They are now among the most widely used insecticides, accounting for ~17% of the total insecticide market.[18,19] Recently, Li's group designed and synthesized a series of neonicotinoid analogues with high insecticidal activity.[20] It should be noted that some of the extremely efficient chemicals have chiral structures. For example, compound **1** has an oxa-bridged structure, while compounds **2** and **3** have one and two asymmetric carbon atoms, respectively (Fig. 1). However, there is very little information with respect to the enantiomeric separation and toxicity of these chiral neonicotinoid insecticides.

This study is the first report on the enantiomeric separation of these three neonicotinoid insecticides using HPLC and SFC. Three commercial polysaccharide-type chiral columns, Chiralpak AD-H, a 3, 5-dimethylphenyl-carbamate

Contract grant sponsor: National Natural Science Foundation of China; Contract grant numbers: 40973077, 20837002, 20707021
Contract grant sponsor: National Basic Research Program of China; Contract grant number: 2009CB421603
Contract grant sponsor: Program for Changjiang Scholars and Innovative Research Teams in Chinese Universities; Contract grant number: IRT 0653
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Received for publication 10 December 2009; Accepted 3 May 2010
DOI: 10.1002/chir.20898
Published online 16 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

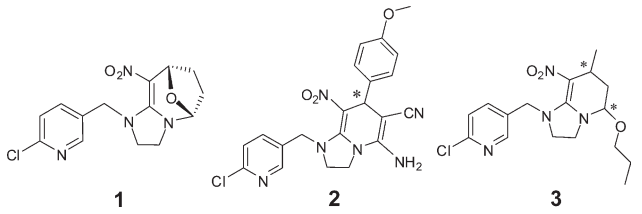

Fig. 1. Structures of neonicotinoid pesticides; *Denotes chiral center.

derivative of amylose, coated onto 5-μm silica gel, Chiralcel OD-H, a 3,5-dimethylphenyl-carbamate derivative of cellulose, coated onto 5-μm silica gel, and Chiralpak IB, a 3,5-dimethylphenyl-carbamate derivative of cellulose, immobilized onto 5-μm silica gel, which have been demonstrated to be highly effective not only for HPLC but also for SFC, were used.[21–25] Results of this study may be useful not only to prepare small quantities of the enantiomers but also to correctly determine their target activities and nontarget toxicities.

MATERIALS AND METHODS

Chemicals and Reagents

Three neonicotinoid insecticides (**1**, **2**, and **3**) with purity >98.0% were kindly donated by the East China University of Science and Technology (Shanghai, China). Stock solutions of all analytes were prepared by dissolution in ethanol at a concentration of ~500 mg L^{-1}. The mobile phases used for chromatography were of HPLC grade and purchased from Tedia Company (Fairfield, OH). Carbon dioxide with a purity of 99.995% was purchased from Jingong Specialty Gas (Hangzhou, China).

Column Information

Three commercial Daicel chiral columns were used in both HPLC and SFC tests. They were Chiralpak AD-H (4.6 × 250 mm ID coated on 5-μm silica gel), Chiralcel OD-H (4.6 × 250 mm ID coated on 5-μm silica gel), and Chiralpak IB (4.6 × 250 mm ID immobilized on 5-μm silica gel).

Apparatus and Chromatographic Conditions

Liquid chromatography was performed on a Jasco LC-2000 series HPLC system (Jasco Corporation, Tokyo, Japan) equipped with a PU-2089 quaternary gradient pump, a mobile phase vacuum degasser, an As-2055 automated microliter syringe with a 25-μl loop, a CO-2060 column thermostat, a UV-2075 detector, a variable-wavelength CD-2095 circular dichroism (CD), and an LC-Net II/ADC signal delivery system. Chromatographic data were acquired and processed by computer-based ChromPass software (version 1.7.403.1, Jasco). For the separation experiments on various columns, the flow rate of mobile phase, the injection volume, and the UV detection wavelength were fixed at 1.0 ml min^{-1}, 5 μl, and 254 nm, respectively. The oven temperature was set at 25°C unless noted otherwise to determine the temperature dependence of enantiomeric separation. In terms of the pressure drop, ethanol proved to be more suitable as the polar modifier than 2-propanol in this study. The initial column screening was conducted at 25°C with a mobile phase of hexane/ethanol (40/60 v/v) at a flow rate of 1.0 ml min^{-1}.

The SFC tests were carried out by a Thar SD-ASFC-2 system (Thar Technologies, Pittsburgh, PA) equipped with a UV/vis-151 detector and a Rheodyne 7410 injector. The flow rate, injection volume, and UV detection wavelength were fixed at 2.0 ml min^{-1}, 5 μl, and 254 nm, respectively. The column temperature was 35°C and the outlet pressure was set at 150 bar.

Chromatographic Characterization

The chromatographic parameters, including retention factor (k), separation factor (α), and resolution (R_s) for the resolved enantiomers of the three neonicotinoid insecticides were calculated and used to evaluate the enantioselectivities of the CSPs. The retention factor (k) was calculated by the following formula:

$$k = \frac{t_R - t_0}{t_0} \quad (1)$$

where t_0 is the column void time determined by recording the first baseline perturbation. t_R is the average retention time of duplicate injections of the analytes taken at the peak maxima. The separation factor α was calculated as:

$$\alpha = \frac{k_2}{k_1} \quad (2)$$

where k_1 and k_2 are the respective retention factors for the first and second elution peaks. The resolution was calculated as:

$$R_s = \frac{1.18(t_2 - t_1)}{(w_{1/2,1} + w_{1/2,2})} \quad (3)$$

where t_1 and t_2 are the peak retention time for the first and second enantiomers eluted, respectively, and $w_{1/2,1}$ and $w_{1/2,2}$ are the half peak widths of the first and second peaks, respectively.

RESULTS AND DISCUSSION

Chiral Separation by HPLC

Enantioseparation is highly specific to CSPs in HPLC analysis. As shown in Tables 1 and 2, baseline resolution of the enantiomers was obtained for both compounds **1** and **2** on both of the coated CSPs. When the ethanol content of the mobile phase was reduced, the values of k increased. However, the corresponding α values were essentially unchanged, resulting in successful separation of **1** and **2** with all *n*-hexane/ethanol combinations used (hexane/ethanol from 10/90 to 50/50). These results suggested that the conformations and adsorption sites of the modified polysaccharide CSPs, as well as the selectand/selector associations between the analytes and the CSPs are probably not affected by the polar components of **1** and **2**.[26] However, stereoselective recognition of **3** on the chiral CSPs was significantly influenced by the ethanol content. As listed in Table 2, satis-

TABLE 1. Chromatographic separation results for 1 and 2 on Chiralpak AD-H column

Compound	1					2				
Hexane/ethanol (v/v)	10/90	20/80	30/70	40/60	50/50	10/90	20/80	30/70	40/60	50/50
k_1	0.51	0.67	0.90	1.33	1.77	0.80	1.05	1.44	2.22	3.41
k_2	2.08	2.66	3.64	5.28	7.36	1.76	1.93	2.65	4.11	6.24
α	4.14	4.05	4.02	3.97	4.16	2.20	1.84	1.83	1.86	1.83
R_s	5.27	5.29	5.33	5.52	6.27	1.70	1.51	1.57	1.85	1.95
CD signal[a]	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−

[a]UV wavelength = 254 nm.

TABLE 2. Chromatographic separation results for 1, 2, and 3 on Chiralcel OD-H column

Compound	1				2				3			
Hexane/ethanol (v/v)	40/60	50/50	60/40	70/30	80/20	40/60	50/50	60/40	70/30	80/20	50/50	60/40	70/30	80/20	90/10
k_1	1.40	1.92	2.98	5.25	11.75	0.69	1.02	1.70	3.26	7.79	0.41	0.64	1.18	2.50	8.54
k_2	1.97	2.65	4.05	7.01	15.46	1.13	1.67	2.82	5.57	13.69	0.49	0.75	1.39	2.90	9.88
k_3	/	/	/	/	/	/		/	/	/	0.96	1.42	2.52	5.02	16.58
k_4	/	/	/	/	/	/	/	/	/	/	1.42	2.07	3.47	7.07	23.12
α_1	1.41	1.38	1.36	1.34	1.32	1.62	1.64	1.67	1.71	1.76	1.18	1.17	1.17	1.16	1.57
α_2	/	/	/	/	/	/	/	/	/	/	1.97	1.89	1.82	1.80	1.68
α_3	/	/	/	/	/	/	/	/	/	/	1.48	1.45	1.37	1.40	1.39
R_{s12}	2.74	2.75	2.77	2.87	3.15	1.98	2.15	2.42	2.84	3.43	0.98	1.09	1.19	1.27	1.49
R_{s23}	/	/	/	/	/	/	/	/	/	/	4.14	4.34	4.52	5.05	5.59
R_{s34}	/	/	/	/	/	/	/	/	/	/	2.60	2.69	2.61	3.39	4.01
CD signal[a]	+/−	+/−	+/−	+/−	+/−	−/+	−/+	−/+	−/+	−/+	+/−/−/+	+/−/−/+	+/−/−/+	+/−/−/+	+/−/−/+

[a]UV wavelength = 254 nm.

factory separation of all four stereoisomers of **3** was only achieved on the Chiralcel OD-H column when hexane/ethanol = 90/10 (v/v) was used as the mobile phase. When the percentage of ethanol in the mobile phase was increased from 20 to 50%, peaks of the first and second eluted isomers were consistently overlapped. On the other hand, when the concentration of ethanol was decreased to 5%, no peak was observed within 100 min (data not shown). Typical separation chromatograms of **1**, **2**, and **3** are shown in Figure 2. In addition, as identified by the CD signals, it is interesting to note that the elution order of the enantiomers of **2** was reversed on Chiralcel OD-H in comparison to Chiralpak AD-H, demonstrating that the chiral recognition interactions of the two CSPs for compound **2** were different.

The chiral selector in Chiralpak IB is of the same nature as that in Chiralcel OD-H, i.e., tris (3,5-dimethylphenylcarbamate) derivatized cellulose. Chiralcel OD-H is made by physical coating of the polymer on a silica gel, whereas the chiral selector in Chiralpak IB is immobilized on the support. It is well known that the immobilization of polysaccharide derivatives on a matrix is an evolutionary approach that uses the universal solvent compatibility of these highly selective CSPs for enantioseparation. Besides the standard mobile phases such as alkane/alcohols, pure alcohols, acetonitrile (ACN) or their mixtures, the so-called nonstandard solvents including dichloromethane (DCM), acetone, tetrahydrofuran, dimethylformamide, or methyl tert-butyl ether (MTBE) can be safely and effectively used as sample diluents. As a result, there is no limitation on the sample injection solvents and racemization of the enantiomers can be avoided if a suitable mobile phase is chosen.[27] More importantly, as the solubilities of **1**, **2**, and **3** in hexane are all extremely limited, the expansion of the mobile phase selection opens up the possibility of improving the performance for both the analytical and preparative resolutions.[27] Table 3 summarizes the chromatographic data achieved on the Chiralpak IB column using six eluents: hexane, ethanol, methanol, ACN, MTBE, and DCM. The results showed that the coated-type Chiralcel OD-H showed a better chiral recognition for both **1** and **2** (Table 2) than the corresponding immobilized-type Chiralpak IB (Table 3) under the same standard conditions. Moreover, the stereoisomers of compound **3** were only partially separated on the Chiralcel IB column with a mixture of *n*-hexane/ethanol = 90/10 (v/v) as the mobile phase (table not shown). However, when the Chiralcel OD-H column was used, nearly baseline separation of compound **3** was obtained under the same conditions (Table 2). This is mainly because the immobilized CSPs are chemically bonded to silica gel through the hydroxyl groups of the polysaccharides, resulting in an alteration in the high-order structure and configuration of the polymers.[28] For the various combinations of solvents listed in Table 3, only a mixture of ethanol and methanol as mobile phase offered successful separation for both the enantiomers of **1** and **2**. Moreover, compound **2** was also enantiomerically separated with a mobile phase of 100% ACN. However, with normal phases and nonstandard solvents such as DCM and MTBE, no enantioseparation was observed, even with a retention time over 60 min (results not shown).

Chiral Separation by SFC

SFC is generally considered to be a normal phase separation technology. A polar organic solvent such as ethanol is

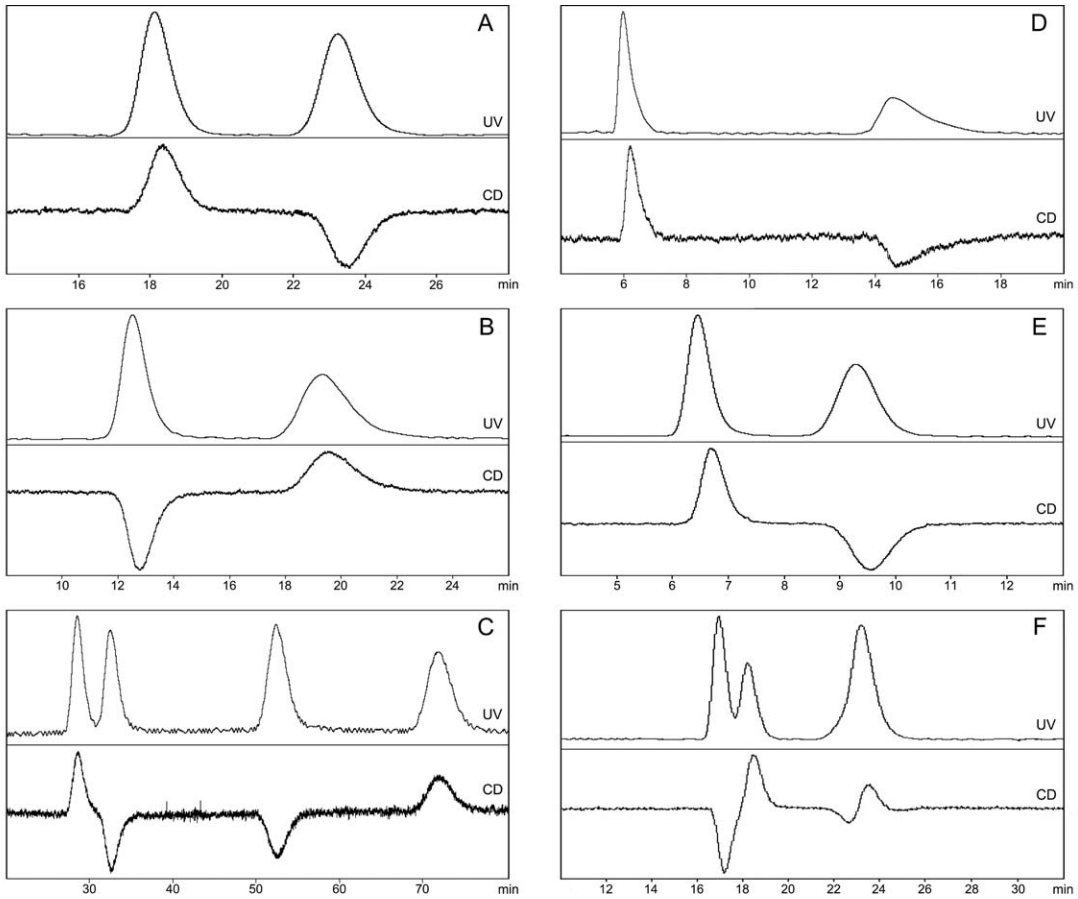

Fig. 2. Optimal chromatograms of compounds **1**, **2**, and **3**. Chromatographic conditions: Chiralcel OD-H column with *n*-hexane/ethanol: **A**: compound **1**, (70/30 v/v); **B**: compound **2**, (70/30 v/v); **C**: compound **3**, (90/10 v/v). Chiralpak AD-H column with *n*-hexane/ethanol; **D**: compound **1**, (40/60 v/v); **E**: compound **2**, (30/70 v/v); **F**: compound **3**, (80/20 v/v). Flow rate: 1.0 ml min^{-1}; column temperature: 25°C.

used as the CO_2 modifier to increase the polarity of the mobile phase. Table 4 gives the chromatographic separation results for SFC. It was found that on the Chiralpak AD-H column, the R_s value of compound **1** was 6.42 for the SFC mode, while the highest R_s value obtained by HPLC was 6.27 (Table 1). Regarding the analysis time, a much shorter elution time was required with SFC than HPLC [less than 15 min compared with 100 min (Fig. 3)]. For the enantiomers of compound **2**, the optimized resolution (R_s) in SFC was 5.23, which was much higher than that (R_s = 1.95) in HPLC. These results suggested that the enantiomers of compound **2** could be baseline separated by either SFC or HPLC with similar retention time (Fig. 3). However, compound **3** could not be chirally resolved on Chiralpak AD-H by either SFC or HPLC. According to the α values, the potential of Chiralcel OD-H for chiral discrimination of compound **1** was poorer when using SFC than when using HPLC (Tables 2 and 4). For instance, the separation factor (α) was 1.08 for SFC when 30% ethanol was used as the polar modifier, while that for HPLC increased to 1.34 in a mobile phase with similar po-

TABLE 3. Chromatographic separation results for 1 and 2 on Chiralcel IB column

Compound	1												2											
Hexane	/	/	/	/	/	/	/	/	30	60	40	60	/	/	/	/	/	/	/	/	30	60	40	60
Ethanol	/	50	100	70	50	30	/	10	/	/	60	40	/	50	100	70	50	30	/	10	/	/	60	40
Methanol	/	/	/	30	50	70	100	/	/	/	/	/	/	/	/	30	50	70	100	/	/	/	/	/
ACN[a]	100	50	/	/	/	/	/	/	/	/	/	/	100	50	/	/	/	/	/	/	/	/	/	/
DCM[b]	/	/	/	/	/	/	/	/	70	40	/	/	/	/	/	/	/	/	/	/	70	40	/	/
MTBE[c]	/	/	/	/	/	/	/	90	/	/	/	/	/	/	/	/	/	/	/	90	/	/	/	/
k_1	0.83	/	2.01	1.03	0.87	0.74	0.66	/	/	/	0.61	3.47	0.17	/	1.36	0.83	0.58	0.50	0.44	/	/	/	1.12	3.08
k_2	0.90	/	2.20	1.15	0.99	0.84	0.74	/	/	/	1.78	3.76	0.29	/	1.69	1.03	0.72	0.62	0.52	/	/	/	1.42	3.70
α	1.08	/	1.09	1.12	1.13	1.13	1.12	/	/	/	2.89	1.08	1.70	/	1.24	1.25	1.24	1.23	1.20	/	/	/	1.27	1.20
R_s	0.86	/	0.58	0.94	1.31	1.23	1.13	/	/	/	0.68	0.61	1.60	/	0.99	1.59	1.52	1.44	1.30	/	/	/	0.93	1.01

[a]Acetonitrile.
[b]Dichloromethane.
[c]Methyl tert-butyl ether.

TABLE 4. Chromatographic separation results for 1, 2, and 3 by SFC

Compound	1			2			3		
Column	AD	OD		AD	OD		OD		
Ethanol (%)	30	30	20	30	30	20	30	20	10
k_1	2.50	3.42	9.29	3.99	4.77	16.78	1.82	5.05	30.38
k_2	5.80	3.69	9.98	9.68	6.15	22.64	2.00	5.37	32.04
k_3	/	/	/	/	/	/	3.02	7.79	46.16
k_4	/	/	/	/	/	/	3.49	9.41	55.1
α_1	2.32	1.08	1.07	2.42	1.29	1.35	1.10	1.06	1.05
α_2	/	/	/	/	/	/	1.51	1.45	1.44
α_3	/	/	/	/	/	/	1.16	1.21	1.19
R_{s12}	6.42	0.92	1.05	5.23	1.70	2.99	0.76	0.76	0.80
R_{s23}	/	/	/	/	/	/	3.15	4.17	4.19
R_{s34}	/	/	/	/	/	/	1.26	2.15	2.04

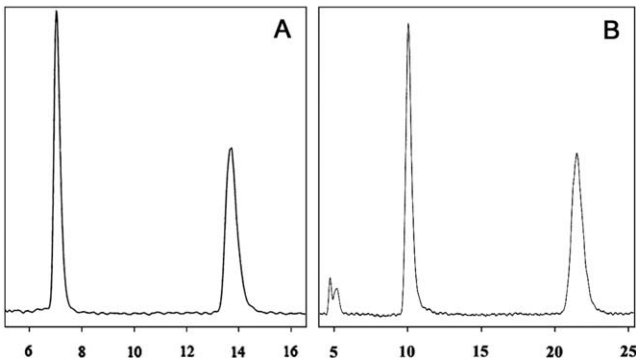

Fig. 3. Optimal chromatograms of compounds **1** and **2**. Chromatographic conditions: **A**: compound **1**, Chiralpak AD-H using CO_2/ethanol (70/30, v/v) as mobile phase; **B**: compound **2** using CO_2/ethanol (80/20, v/v) as mobile phase. Flow rate: 2.0 ml min^{-1}; column temperature: 35°C.

TABLE 5. Effect of temperature on the enantiomeric separation of 1, 2, and 3 by HPLC on the Chiralcel OD-H column

Compound	1					2					3				
Temperature (°C)	15	20	25	30	35	15	20	25	30	35	15	20	25	30	35
k_1	5.88	5.65	5.25	4.62	4.18	3.83	3.52	3.26	2.99	2.77	1.37	1.27	1.18	1.09	1.01
k_2	8.11	7.61	7.34	6.15	5.53	6.96	6.19	5.57	4.95	4.43	1.65	1.49	1.39	1.24	1.13
k_3	/	/	/	/	/	/	/	/	/	/	3.07	2.74	2.52	2.21	1.94
k_4	/	/	/	/	/	/	/	/	/	/	4.42	3.92	3.47	3.10	2.77
α_1	1.38	1.32	1.34	1.33	1.32	1.82	1.76	1.71	1.66	1.60	1.20	1.17	1.17	1.14	1.12
α_2	/	/	/	/	/	/	/	/	/	/	1.86	1.84	1.82	1.78	1.72
α_3	/	/	/	/	/	/	/	/	/	/	1.44	1.43	1.37	1.40	1.43
R_{s12}	2.67	2.82	2.87	3.12	3.20	2.45	2.66	2.84	2.97	3.08	1.18	0.94	1.19	0.81	0.86
R_{s23}	/	/	/	/	/	/	/	/	/	/	4.19	4.08	4.52	4.33	5.11
R_{s34}	/	/	/	/	/	/	/	/	/	/	2.62	2.80	2.61	3.02	3.07

TABLE 6. Effect of temperature on the enantiomeric separation of 1, 2, and 3 by SFC on the Chiralcel OD-H column

Compound	1			2			3		
Temperature (°C)	35	40	45	35	40	45	35	40	45
k_1	2.97	2.85	3.10	3.67	3.85	4.14	1.26	1.24	1.16
k_2	3.14	3.05	3.36	5.06	5.11	5.37	1.35	1.32	1.30
k_3	/	/	/	/	/	/	2.18	2.11	2.20
k_4	/	/	/	/	/	/	2.67	2.58	2.55
α_1	1.06	1.07	1.08	1.38	1.33	1.30	1.07	1.06	1.12
α_2	/	/	/	/	/	/	1.61	1.60	1.69
α_3	/	/	/	/	/	/	1.22	1.22	1.16
R_{s12}	0.79	0.81	0.83	2.77	2.54	2.15	0.61	0.44	0.91
R_{s23}	/	/	/	/	/	/	4.40	2.82	3.67
R_{s34}	/	/	/	/	/	/	2.14	1.29	1.11

TABLE 7. Summary of enthalpy, entropy, and T_{iso}

	HPLC				SFC			
Compound	$\Delta\Delta H^\circ$ (J mol^{-1})	$\Delta\Delta S^\circ$ (J mol^{-1} K^{-1})	R_2	T_{iso} (°C)	$\Delta\Delta H^\circ$ (J mol^{-1})	$\Delta\Delta S^\circ$ (J mol^{-1} K^{-1})	R_2	T_{iso} (°C)
1	/	/	/	/	2026.6	7.04	1.00	14.86
2	−4660.50	−11.20	0.997	143.11	−4872.34	−13.17	0.99	96.93

larity. Compound **2** was found to be satisfactorily enantiomerically separated by both HPLC and SFC when using Chiralcel OD-H as the chiral selector, with the chiral recognition capability of the former method being slightly better (R_s = 3.43 vs. 2.99; Tables 2 and 4). However, the stereoisomers of **3** were only partially resolved on the Chiralcel OD-H by SFC (Table 4). For the Chiralcel IB column, no effective separation of the stereoisomers of the three analytes was observed under SFC conditions, which did not appear to be any better than those of HPLC.

In summary, SFC had some advantages over HPLC in the chiral separation of the neonicotinoid insecticides we studied. First, SFC separation needed shorter retention time than HPLC, which greatly reduces the amount of organic solvent required, and is helpful for environmental safety. Second, separation of the enantiomers of 1 and 2 on Chiralpak AD-H was more efficient by SFC than by HPLC, especially for the preparation of optically pure isomers.

Effect of Temperature

Temperature is an important parameter in understanding the chromatographic behaviors of the analytes as well as the characteristics of the stationary phase. Changes in the column temperature can also be used to improve the enantioselectivity of the CSPs. Given its substantially better performance, Chiralcel OD-H was chosen to determine the effects of temperature in both HPLC and SFC modes. In the HPLC tests, the column temperature was set from 15 to 35°C at intervals of 5°C. In the case of SFC, only three temperatures, that is, 35, 40, and 45°C were used. The ethanol content was maintained at 30% for both HPLC and SFC analyses. The values of k, α, and R_s at various temperatures are shown in Table 5 for HPLC and Table 6 for SFC.

Data in Table 5 show that the values of both k and α for compounds **1** and **2** decreased notably with an increase in column temperature, while the values of R_s increased. The effect of temperature on chiral discrimination of the CSPs was more complex for **3** than for **1** and **2**. For example, the maximum R_s values were obtained at 25, 35, and 35°C for R_{s12}, R_{s23}, and R_{s34}, respectively (Table 5). In addition, the separation factors (α) did not always decrease with an increased oven temperature. However, the first and second eluted isomers did not separate successfully at any of the temperatures used.

In the SFC test, the influence of temperature on the chiral separation of **1** was extremely slight, especially in view of the values of α and R_s. For compound **2**, the retention time increased with an increased temperature, which was the opposite result to that for HPLC. A similar result was obtained for the enantiomeric separation of proline derivatives using SFC.[29] This phenomenon may be ascribed to the fact that although the pressure is kept constant as the temperature increases, the density decreases, resulting in reduced solvating power of the mobile phase, hence increasing the retention of analytes. The α and R_s values of **2** decreased slightly when the column temperature was increased, with a better selectivity at a lower temperature. In the cases where poor separation was achieved for compound **3**, the values of k and α changed slightly with an increase in temperature. However, the effect of temperature was more significant on the corresponding R_s values.

Temperature can affect chiral separation in at least two ways. One is its effect on the viscosity and diffusion coefficient of the solute. The other is the thermodynamic effect that changes the separation factor (α).[30] Generally, the former is small enough to be negligible, whereas the latter is more important and can be investigated using the following three equations:

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \phi \tag{4}$$

and

$$\ln \alpha = -\frac{\Delta\Delta H^\circ}{RT} + \frac{\Delta\Delta S^\circ}{R} \tag{5}$$

and

$$T_{iso} = \frac{\Delta\Delta H^\circ}{\Delta\Delta S^\circ} \tag{6}$$

In the above equations, ϕ, R, and T mean the phase ratio, gas constant, and absolute temperature, respectively. ΔH° and ΔS° are the standard transfer enthalpy and entropy of the analyte from the mobile phase to the stationary phase; and $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ are the differences ($\Delta H_2 - \Delta H_1$) and ($\Delta S_2 - \Delta S_1$), respectively. When the enthalpy and entropy contributions become equal, the selectivity equals one and coelution occurs. The temperature at which coelution occurs is called the isoelution temperature (T_{iso}).

Under HPLC conditions, significant deviation from linearity was observed for both **1** and **3** (data not shown), it may due to the conformational change of the CSP.[31] Only **2** gave a linear response ($R_2 \geq 0.99$). Under SFC conditions, **1** and **2** showed a linear relationship. The calculated results for $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ for HPLC and SFC are tabulated in Table 7. In the case of HPLC, the T_{iso} of compound **2** was above the range of temperatures examined, suggesting that enantioseparation of **2** was enthalpy controlled, and could be better achieved at a lower temperature. Under SFC conditions, compounds **1** and **2** had linear responses. The T_{iso} obtained for compound **1** was below and relatively close to the temperatures assayed. Its separation was entropy controlled. On the other hand, the high T_{iso} of compound **2** indicated an enthalpy-controlled separation, suggesting that better separation would occur at lower temperatures.[32]

CONCLUSIONS

Results of this study demonstrated that three chiral neonicotinoid insecticides (**1**, **2**, and **3**) could be successfully enantiomerically separated on a Chiralcel OD column by HPLC. For the two compounds with only one asymmetric center, i.e., **1** and **2**, Chiralpak AD and Chiralcel IB also worked. Moreover, satisfactory chiral separations of **1** and **2** were also obtained by SFC with lower retention time than by HPLC, so SFC seems to be suitable for individual enantiomer preparation. Overall, the established method could be used for preparing small amounts of pure enantiomers of the chiral neonicotinoid insecticides studied.

ACKNOWLEDGMENTS

The authors thank Xusheng, Shao of the East China University for kindly providing the three neonicotinoid insecticides.

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