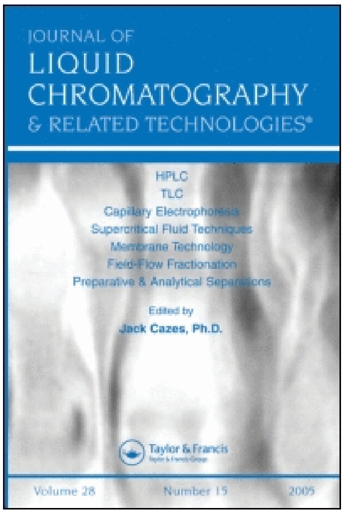

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ABSTRACT

The direct optical resolution of five chiral pesticides on a cellulose *tris*-3,5-dimethylphenyl carbamate stationary phase (Chiralcel OD, 250×4.6 mm) under reversed-phase conditions is described. The influence of organic modifiers, pH, and temperature was investigated. The two mobile phases used were water/methanol and water/acetonitrile. Of five chiral pesticides investigated, the three pesticides were observed

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to obtain the base separation under the suitable conditions and the other two were observed to be partially resolved. The values of α (1.04–6.65) and R_s (0.63–13.16) have been reported.

Key Words: Direct optical resolution; Chiral pesticides; HPLC; Reversed phase conditions.

INTRODUCTION

The importance of separation of chiral compounds has been widely realized. Owing to the enantioselective interaction with enzymes, cellular receptors, or other chiral biological molecules,[1] many enantiomeric pharmaceuticals often display different activities in biological systems, and so have different rates of metabolism and environmental fate. There are about 25% chiral pesticides in the world commercial pesticide markets, and most of these chiral pesticides are still sold in the form of racemates.[2] Generally, enantiomers of chiral pesticides have different bioactivity, toxicality, and degradation rates. To avoid side effects caused by one of enantiomers, it is desirable to use drugs in optical pure forms.[3] For example, metalaxyl, a systemic, benzenoid fungicide, used as a foliar spray for tropical and subtropical crops or as a soil treatment for control of soil-borne pathogens or as a seed treatment to control downy mildews, is mainly sold as racemates. But studies showed that the efenoxam (R) isomer is capable of more effectiveness and easier degradation than its (S) isomer.[4,5] So it is of importance to develop its (R) isomer to reduce the pesticide residue and to protect environment. Triadimefon, also a systemic fungicide, provides better powdery mildew control than most other fungicides. Myclobutanilis is used for the control of brown rot, leaf spot, and powdery mildew on peppers for preharvest management. Fenoxaprop-ethyl is a kind of herbicide. The above four pesticides all have a chiral center and are mainly sold as racemates. Hexythiazox is a kind of insecticide to get rid of the eggs and young pests in the plants. Hexythiazox has two chiral carbon atoms, but it was produced as reverse form, thus it also has two enantiomers. The above five chiral pesticides play important roles in killing plant illnesses and pests in the world agriculture. So it is of urgency and necessity to establish an effective method to separate the above five chiral pesticides to improve their effectiveness, decrease the pesticide residue, and protect the environment.

Chromatographic enantioseparation, particularly by high performance liquid chromatography (HPLC) using a chiral stationary phase (CSP), has become the most powerful method available for obtaining both enantiomers and determining their enantiomeric purities.[6] Among them, cellulose *tris*-3,5-dimethylphenyl carbamate (CDMPC) CSP has been found to be a major tool of analysis. Though some of the above pesticides have been reported to be separated by CDMPC under normal phase conditions such as metalaxyl, triadimefon, and hexythiazox,[7–9] to our knowledge there is no study in the literature describing a complete evaluation of CDMPC under reversed-phase for the resolution of the drugs selected for the present investigation (Fig. 1).

EXPERIMENTAL

Reagents and Relevant Materials

All reagents were of analytical grade. Methanol and acetonitrile were obtained from Tianjin WeiSi Experimental Ltd. Company. Purified water was purchased from Wahaha purified water factory. Metalaxyl, triadimefon, and myclobutanil were obtained from Heben Herbicide Chemicals Ltd. Company, Zhejiang Province. Hexythiazox was obtained from WeiErDa Chemicals Ltd. Company, Zhejiang Province. Henoxaprop-ethyl was obtained from Hangzhou YuLong Chemicals Co. Ltd. Hydrochloric acid and acetic acid were purchased from chemical reagents six factory, Tianjin city. The pH Meter was purchased from Shanghai Leici Instrumental Factory.

HPLC Conditions

HPLC was carried out on an Agilent 1100 with quaternary-pump system, mobile phase vacuum degassers, and an ultraviolet detector. The samples were injected with amounts of 10 μL. The detection of metalaxyl and hexythiazox was performed at 230 nm; the detection of myclobutanil was performed at

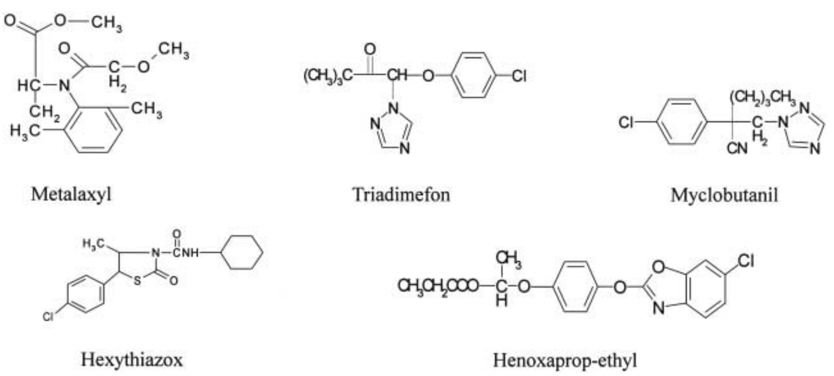


Figure 1. The structures of the chiral pesticides in this study.

225 nm; the detection of triadimefon and henoxaprop-ethyl was performed at 254 nm. The mobile phases used were different mixtures of methanol/water or acetonitrile/water. The flow rate of the mobile phase was 0.8 mL/min. All separations, except the experiment for the influence of temperature, were carried out at room temperature. Data was obtained and processed from Agilent ChemStation. The retention factors (k') were determined as $k' = (t_R - t_0)/t_0$. The enatioselectivity factors (α) were calculated as $\alpha = k'_2/k'_1$, where k'_2 and k'_1 were retention factors for the first and second eluting enantiomer, respectively. The resolution factors (R_s) were calculated as $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 were base widths for the first and second eluting enantiomer, respectively. The chromatographic parameters values are the mean of three experiments.

CSP Column

The HPLC column used was cellulose *tris*-3,5-dimethylphenyl carbamate (Chiralcel OD). The column was 250 × 4.6 mm I.D., with the enantioselective phase coated onto a 5 μm silica-gel substrate. The Chiralcel OD was prepared and packed following the guidance of the literature[10,11] in the laboratory.

RESULTS AND DISCUSSION

Effect of Mobile Phase Composition on Enantioselective Separations

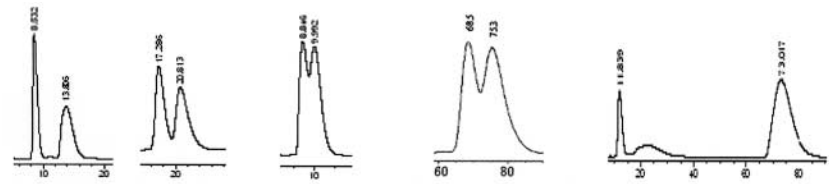
Different mobile phase compositions, such as methanol/water mixtures and acetonitrile/water mixtures, were investigated. The chromatographic results of the chiral separation with methanol/water and acetonitrile/water are listed in Table 1. For the above five pesticides, better resolution generally can be obtained in methanol/water than in the acetonitrile/water. The enatioselectivity factor (α) and resolution factor (R_s) are higher in methanol/water than in acetonitrile/water, and retention time in methanol/water is also higher than in the acetonitrile/water. Metalaxyl and myclobutanil can be completely separated both in methanol/water and acetonitrile/water with suitable conditions. Triadimefon, fenoxaprop-ethyl, and hexythiazox can only be separated in methanol/water (Table 1, Figs. 2 and 3). Since methanol and acetonitrile possess different hydrogen bonding capability, it can be inferred that hydrogen bonding might play an important role in the enantioselectivity on Chiralpak OD under RP conditions.

The influence of the water content in the mobile phase on the chiral separation was also investigated. The results demonstrated that α and R_s increased with the water content of the mobile phase. The water content

Table 1. The chromatographic separation results of the chiral pesticides with methanol/water and acetonitrile/water as mobile phase.

Mobile phase	Metalaxyl			Myclobutanil			Triadimefon			Fenoxaprop-ethyl			Hexythiazox		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
Methanol/water[a]															
90 : 10	3.59	1.46	3.67	4.52	1.18	1.76	3.3	1.06	0.86	7.1	1.04	0.63	3.8	1.84	6.31
80 : 20	4.51	1.54	3.95	7.06	1.2	1.82	4.91	1.12	1.15	18.28	1.09	1.24	5.67	3.23	11.22
70 : 30	6.88	1.75	4.07	14.68	1.22	1.86	7.04	1.15	1.16	61.32	1.1	1.24	10.02	6.65	13.16
65 : 35	8.52	1.75	4.15	20.94	1.22	1.93	14.08	1.17	1.41	116.44	1.11	1.3	/	/	/
60 : 40	14.19	2.02	5.28	39.75	1.23	1.95	23.57	1.2	1.99	/	/	/	/	/	/
55 : 45	20.72	2.05	5.7	67.03	1.25	1.96	46.83	1.25	2.25	/	/	/	/	/	/
Acetonitrile/water[b]															
90 : 10	2.65	1.25	1.98	3.00	1.13	1.27									
80 : 20	2.55	1.35	2.98	3.17	1.16	1.47									
70 : 30	2.83	1.41	3.12	3.90	1.19	1.79									
60 : 40	3.44	1.48	3.51	5.74	1.24	2.05									
50 : 50	4.65	1.55	4.37	10.22	1.28	2.75									

[a]Data of fenoxaprop-ethyl and hexythiazox can only be obtained with the biggest ratio of 65 : 35 and 70 : 30 for the mobile phase of methanol/water because the retention time of enantiomers is too long (>150 min), flow rate is 0.8 mL/min; room temperature.
[b]Flow rate is 0.8 mL/min, room temperature.



2a (Metalaxyl) **2b**(Myclobutanil) **2c**(Triadimefon) **2d**(Fenoxaprop-ethyl) **2e**(Hexythiazox)

Figure 2. The chromatograms of pesticides in methanol/water (70 : 30 v/v, flow rate is 0.8 mL/min, room temperature).

can only be increased to 45%, 50% for methanol and acetonitrile, respectively, because of the limitation of the column pressure. The effect of water on enantioselectivity in the range from 10% to 50% could be caused by an alteration of the structures of higher order of the cellulose *tris*-3,5-bimethylapheny carbamate.

Influence of pH on Enantioselective Separations

The influence of pH was investigated over a range from 2 to 6 using acid (hydrochloric acid and acetic acid) to adjust pH. However, no significant change in retention and resolution was observed. This might be due to the fact that the pesticides investigated are neutral. Therefore, in further experiments, no buffer was used. Table 2 shows the result.

Influence of Column Temperature on the Chiral Separation

The influence of column temperature was investigated over a range of 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, while maintaining the mobile phase as methanol/water (80 : 20 v/v). Table 3 shows the results of the chiral separation.

3a (Metalaxyl) **3b** (Myclobutanil)

Figure 3. The chromatograms of pesticides in acetonitrile/water (70 : 30 v/v, flow rate is 0.8 mL/min, room temperature).

Table 2. Influence of pH on enantioselective separations of five chiral pesticides.

pH	Metalaxyl			Myclobutanil			Triadimefon			Fenoxaprop-ethyl			Hexythiazox		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
6	4.74	1.62	4.62	7.09	1.21	2.03	6.47	1.19	1.69	5.84	1.29	1.02	5.82	3.64	9.09
5	4.74	1.66	4.67	7.00	1.22	2.08	5.02	1.14	1.5	17.83	1.08	1.16	5.74	3.56	10.31
4	4.66	1.62	4.75	6.84	1.21	2.09	5.15	1.14	1.49	19.76	1.09	1.19	5.78	3.69	10.49
3	4.89	1.65	4.76	7.18	1.21	2.06	5.25	1.14	1.48	20.68	1.09	1.24	5.87	3.71	10.53
2.5	4.58	1.56	4.3	6.26	1.19	1.96	4.93	1.13	1.32	22.37	1.1	1.26	7.20	4.06	10.54

Note: Methanol/water 80 : 20 v/v, flow rate is 0.8 mL/min, room temperature.

Table 3. Influence of column temperature on the chiral separation.

Temperature (°C)	Metalaxyl			Myclobutanil			Triadimefon			Fenoxaprop-ethyl			Hexythiazox		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
0	7.93	1.93	5.31	11.33	1.24	1.89	7.40	1.19	1.61	37.16	1.14	1.52	7.94	4.32	9.78
10	5.63	1.72	4.7	8.72	1.22	1.86	6.45	1.16	1.49	27.31	1.11	1.21	8.06	3.73	9.76
20	4.74	1.54	4.14	6.73	1.2	1.86	5.15	1.12	1.2	17.91	1.08	1.06	6.00	3.2	9.64
30	4.14	1.39	3.34	5.53	1.16	1.66	4.51	1.09	0.99	13.51	1.05	0.73	5.22	2.76	9.57
40	3.81	1.29	2.53	4.85	1.13	1.31	4.07	1.06	0.66	10.67	1	0	4.51	2.31	8.47
50	3.47	1.19	1.7	4.31	1.1	1.07	3.88	1	0	8.92	1	0	4.61	2.12	8.29

Note: Methanol/water 80 : 20 v/v, flow rate is 0.8 mL/m.

The results show that the enantioselectivity of the chiral pesticides increased when the column temperature decreased. However, for the compounds such as fenoxaprop-ethyl and triadimefon showing low resolution, the effect of temperature is limited. Base separation of both pesticides still can not be obtained at 0°C. So, the decrease in temperature can only partially benefit chiral resolution on Chiralpak OD under RP conditions. Figure 4a and b show the chromatographic separation of metalaxyl and triadimefon respectively.

CONCLUSION

It is possible to use Chiralpak OD columns with aqueous eluents to separate the chiral pesticides. The use under RP conditions enhances the applicability of this CSP because it enables the chiral separation of many compounds, which cannot be resolved under NP conditions.

The substitution of methanol for acetonitrile in the aqueous eluent did dramatically change the general separation characteristics. This points to an important role of hydrogen bonding as retention mechanism on Chiralpak OD under RP conditions. The use of methanol/water instead of acetonitrile/water can improve the chiral separation.

For the five chiral pesticides' racemates, enantioselectivity increased with increasing concentration of water in the eluent, up to 45%, 50% for methanol and acetonitrile, respectively.

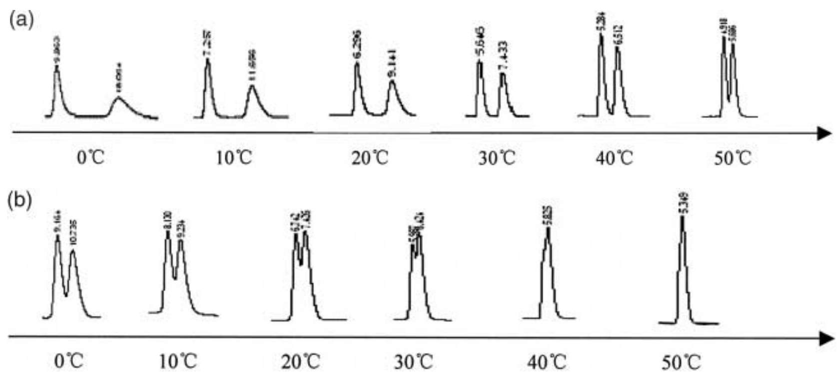


Figure 4. (a) The chromatograms of metalaxyl with different column temperatures. (Mobile phase: methanol/water 80 : 20 vv, flow rate: 0.8 mL/min.) (b) The chromatograms of triadimefon with different column temperatures. (Mobile phase: methanol/water 80 : 20 vv, flow rate: 0.8 mL/min.)

It was observed that the influence of pH has no significant change in retention and resolution. This might be due to the fact that the pesticides investigated are neutral.

With the decrease in column temperature, the enantioselectivity was observed to increase. But the decrease in column temperature can only partially benefit chiral resolution on Chiralpak OD under PR condition for the compounds showing low resolution.

A RP-HPLC method for the separation of chiral pesticides was successfully developed and, thus, demonstrated the aptitude of Chiralpak OD columns for RP chromatography.

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