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Separation of Bifenthrin Enantiomers by Chiral HPLC and Determination of Their Toxicity to Aquatic Organism

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ABSTRACT

In this study, we separate enantiomers of bifenthrin, a pyrethroid insecticide, by using high performance liquid chromatography with a chiral stationary phase (CPS) column. Toxicity tests of bifenthrin enantiomers were performed with Daphnia (water flea, *Daphnia pulex*), tilapia fish (*Tilapia* spp.) and carp fish (*Cyprinus carpio*). Two enantiomers of bifenthrin were separated successfully by injecting 20 μL of bifenthrin 1000 mg/L standard solution into a Sumichiral OA-2500-I column. The mobile phase solvent system was composed of *n*-hexane, isopropanol, and ethanol in a ratio of 99.8/0.06/0.14, and the flow rate was 1.0 mL/min. Both enantiomers were eluted within 20 min. Two separated enantiomers, (-)-bifenthrin and (+)-bifenthrin, were identified by gas chromatography with mass spectrometry detector (GC-MSD) and polarimeter. Each enantiomer of bifenthrin and the standard bifenthrin solution showed the same fragmentation pattern spectrum. The fragment ions were *m/z* 115, *m/z* 141, *m/z* 165, *m/z* 181, and *m/z* 422. The specific rotations $[\alpha]^{20}_{365}$ of (-)-bifenthrin and (+)-bifenthrin were -66.41° and +69.63°, respectively. In the toxicity study, the LC_{50} (12 hr) of (-)-bifenthrin and (+)-bifenthrin for daphina were 2.1 μg/L and 28.9 μg/L, respectively. On the other hand, the result of fish toxicity indicated the LC_{50} (96 hr) of (-)-bifenthrin and (+)-bifenthrin for carp (*C. carpio*) were 0.99 and 2.08 μg/L, respectively, and for tilapia (*Tilapia* spp.) were 0.19 and 0.80 μg/L, respectively. The study of biological activity showed that the toxicity of (-)-bifenthrin was 10-fold greater than (+)-bifenthrin for Daphnia, and 2-fold greater for fish. This study provided improved methods for separating enantiomers of bifenthrin and assessing hazard of individual enantiomers toward non-target aquatic fauna.

Key words: HPLC, bifenthrin, biological activity, enantiomers

INTRODUCTION

Most pyrethroid insecticides are relatively non-toxic to birds or mammals. In recent years, synthetic pyrethroid insecticides have been extensively used for agricultural spraying and public health[1,2]. However, their high toxicity to fish and other aquatic organisms is one of the problems in using pyrethroid insecticides. It is well known that pyrethroid insecticides have different optical isomers, with each isomer having significantly different biological activity. Alzogaray *et al.*[3] found that *cis*-permethrin was about 20 times more toxic than *trans*-permethrin to blood-sucking insect. Elliott *et al.*[4] also reported that the toxicity of (S)-deltamethrin to houseflies was 2 to 2.5-fold greater than that of (RS)-deltamethrin.

In order to reduce the effect on non-target aquatic organisms like daphnia and fish, the third-generation pyrethroid insecticide such as bioallethrin or alpha-cypermethrin, have been developed. These also have better effect on killing target insects. According to Yameogo *et al.*[5], toxicity for tilapia fish of etofenprox®, a pseudopyrethroid, is far below than that of permethrin.

It is meaningful to separate more insecticidal optical isomers and those with fewer hazards from non-target organisms. Chromatography effectively separates isomers of pyrethroid insecticide. Studies have shown, separation of pyrethroid isomers done successfully by changing the mobile and stationary phases of HPLC. Okamoto *et al.*[6] successfully separated phenothrin isomers by the silica gel coated column. Cayley *et al.*[7] showed the separation of *cis*- and *trans*-cypermethrin by using a covalently bonded Pirkle type 1-A stationary phase column. However, the separation of enantiomers is more difficult to achieve, due to similar chemical properties, and there are fewer reports are available on this aspect. Chapman[8] separated some of the enantiomers of fenpropanate, cypermethrin, cyfluthrin and fenvalerate by using a NH_2-bonded Pirkle type 1-A column with normal phase mobile phases. Doi *et al.*[9] demonstrated the separation of 4 optical isomers of teramethrin and resmethrin by an OA-2000 chiral column manufactured by Sumika Chemical Analysis Service Inc. Oi *et al.*[10] used an OA-2500 series CSPs column to successfully separate 9 different pyrethroid insecticides. Almost at the same time Lisseter and Hambling[11] also provided the results of separation for many pyrethroid insecticides, although the resolution was not as good as Oi *et al.*[10].

In this research, we described a modified faster procedure for separating pyrethroid insecticide bifenthrin by using an OA-2500-I CSPs column and a normal phase HPLC. In addition, the toxicity of the separated enantiomers of bifenthrin toward daphnia and fish was also evaluated for the environmental hazard assessment.

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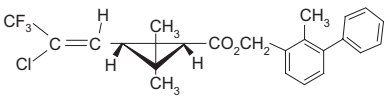
MATERIALS AND METHODS

I. *Chemicals*

Insecticide bifenthrin (2-methylbiphenyl-3-ylmethyl(Z)-(1RS,3RS)-3-(2-chloro-3,3,3,-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate) was purchased from Merck Co. (Darmstadt, Germany) with chemical properties as shown in Table 1[12]. *n*-Hexane, 1,2-dichloroethane, ethanol, isopropanol and acetone purchased from Merck Co. (Darmstadt, Germany) were of HPLC grade. The stock solutions (1000 mg/L) were prepared in *n*-hexane and kept in cold storage.

Table 1. Selected properties of bifenthrin[12]

	Bifenthrin
Molecular structure	$C_{23}H_{22}ClF_3O_2$
IUPAC name	2-methylbiphenyl-3-ylmethyl(Z)-(1RS,3RS) -3-(2-chloro-3,3,3,-trifluoroprop-1-enyl)-2,2 –dimethylcyclopropanecarboxylate
Structural formula	
Molecular weight	422.9
Solubility	In water 0.0001 g/L (20°C) In acetone 1250 g/L (20°C)
Melting point	51~66°C
Vapor pressure	0.024 mPa (25°C)
Kow	logP >6

II. *Separation of Bifenthrin Enantiomers by HPLC*

To separate the optical isomers (B1 and B2) of bifenthrin, standard pure bifenthrin solution at 100 mg/L was prepared as the sample for injection of HPLC. After injecting the sample, the separated optical isomers were collected in brown vials. Each optical isomer sample was concentrated and then reconstituted in *n*-hexane for further analysis.

The isolation and quantification of the separated optical isomer employed the high performance liquid chromatography (HP1100, Hewlett Packard Co., USA), which was equipped with a Sumichiral OA-2500I (5 μm) (250×4.6 mm I.D.) column (Sumica Chemical Analysis Service, Japan) and a photodiode array detector (PAD). The injection volume was 20 μL, and the detection wavelength was set at 230 nm. The mobile phase was composed of isopropanol and ethanol, and the flow rate was 1.0 mL/min.

III. *Identify the Enantiomers by GC-MSD*

To confirm the separated isomers, a GC-MSD (Saturn 2100, Varian Inc., USA) equipped with DB-5 fused silica capillary column was used. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. Injection mode was splitless. The injection volume was 1 μL. Injection port temperature was set at 300°C. The interface, ion source and quadruapole temperatures were 280, 230 and 106°C, respectively. The oven temperature was programmed initially set at 80°C, and then increased to 200°C at 30°C/min and to 300°C at 10°C/min.

To determine the polarities of the separated isomer samples, a polarimeter (Model P341LC, Perkin-Elmer Co., USA) with OROT scan mode (angle of rotation mode) was used. The rotary range was ± 85°. The wavelength of polarimeter was set at 365 nm and the temperature was set at 20.0°C.

IV. *Biological Toxicity Test*

In order to understand the toxicity on aquatic fauna, the static exposure method was used for bioassay[13]. Ten *Daphnia* (*Daphnia pulex*) within 24 hr of birth were put into a 250-mL beaker with 100 mL of tap water. To avoid the possible toxicity caused by the solvent, 0.25, 0.5 or 1 mL of acetone was added to the sample water and the mortality of daphnia was then measured. To determine the LC_{50} of separated isomers for Daphnia, (-)-bifenthrin was added into the sample water at final concentrations of 1, 3, 4, 5 or 7 μg/L, whereas (+)-bifenthrin was added at final concentrations of 10, 30, 40, 50 or 70 μg/L. The blank had 250 μL of acetone added. Death of daphnia was defined as the absence of coordinated movement within 15 sec after being prodded with a plastic dropper or beating the beaker with a glass stick. In addition, 10 Carp fish (*Cyprinus carpio*) or Tilapia fish (*Tilapia* spp.), around 1 or 2 months old with average body length between 1.4 and 2.0 cm, were put into a 10-L beaker with 5 L of tap water sample. Different serial concentrations of (-)-bifenthrin (0.2, 0.4, 0.8, 1.6, or 2.5 μg/L) and (+)-bifenthrin (0.5, 1.0, 2.2, 3.6, or 9.6 μg/L) were added to the beakers with the Carp for determining the LC_{50}. In addition, (-)- or (+)-bifenthrin was added into the beakers with Tilapia fishes at given final concentrations (0.2, 0.3, 0.4, 0.5 or 0.6 μg/L, respectively, for (-)-bifenthrin, and 0.18, 0.4, 0.9, 3.6 or 9.0, respectively, for (+)-bifenthrin) for the same purpose. The blank had 0.5 mL of acetone added. Motility of fish was defined as the gills or fins having stopped moving or the fish bodies having turned over. Each treatment described above was performed in triplicate. The LC_{50} was calculated by using the Probit Method program.

RESULTS AND DISCUSSION

I. *Separation of Bifenthrin Optical Isomers by Chiral HPLC*

Two bifenthrin optical isomers were separated by using a Sumichiral OA-2500-I column equipped in the HPLC. The mobile phase composed of *n*-hexane, isopropanol, and ethanol in a ratio of 99.8/0.1/0.1 was tested. The optical isomers of bifenthrin were successfully distributed into 2 isomers B1 and B2 with retention time of 31.645 and 34.123

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005

Table 2. Resolution (Rs), capacity factor (k') and separation factor (α) of (+) and (-)-bifenthrin in Sumichiral OA-2500-I column

Peak no.	Optical property	k'_1/k'_2	Separation factor (α)	Resolution (Rs)
B1/B2	(-)/(+)	1.59/1.84	1.16	3.00

Table 3. Acute toxicity (LC_{50}) of (-) and (+)-bifenthrin in daphnia, carp and tilapia

Bifenthrin	Daphnia		Carp		Tilapia	
	4 hr (μg/L)	12 hr (μg/L)	48 hr (μg/L)	96 hr (μg/L)	48 hr (μg/L)	96 hr (μg/L)
(-)	4.16 ± 0.26[a]	2.13 ± 0.50	1.31 ± 0.30	0.99 ± 0.29	1.52 ± 0.79	0.19 ± 0.18
(+)	88.12 ± 1.40	28.97 ± 0.26	24.03 ± 4.62	2.08 ± 0.43	16.59 ± 5.41	0.80 ± 0.09

[a]Data present as mean ± S.D. (n = 3).

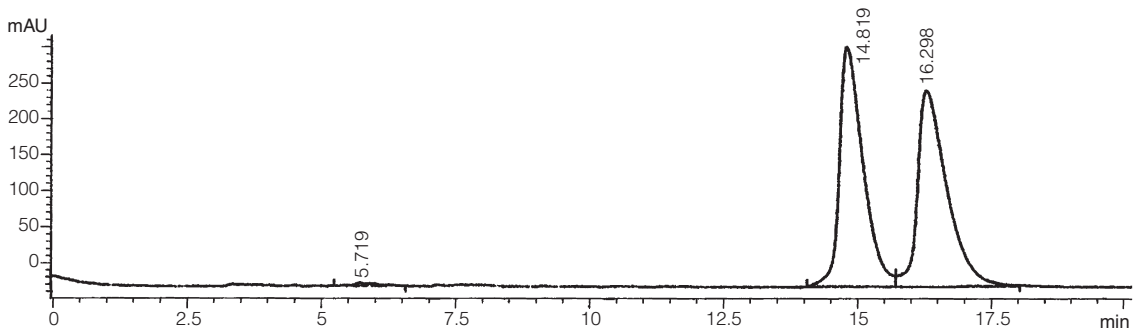


Figure 1. HPLC chromatogram of 1000 mg/L bifenthrin standard solution separated by Sumichiral OA-2500-I column. The mobile phase is *n*-hexane/isopropanol/methanol (99.8/0.06/0.14). The retention times of (-)-bifenthrin and (+)-bifenthrin are 14.819 min and 16.298 min, respectively.

min (chromatogram not shown), respectively. In addition, a better mobile phase system consisting of *n*-hexane, isopropanol, and ethanol in a ratio of 99.8/0.06/0.14 was used to reduce time. The results presented in Figure 1 show that the time was reduced by almost 50%. The isomers of bifenthrin, B1 with retention time of 14.819 min, and B2 with retention time of 16.298 min, were separated with an initial concentration 10-fold (1000 mg/L, 20 μL) greater than the previous method. Because resolution is a quantitative measure of the success of a separation, we calculated the resolution (Rs), capacity factor (k') and separation factor (α) of B1 and B2 to prove the quantity performed in this study (Table 2). Oi *et al.*[10] used the same column to successfully separate other pyrethroid isomers, but the maximum injecting concentration was limited within 100 mg/L. In this study, we found a more efficient method to separate the isomers of bifenthrin and to investigate the third-generation pyrethroid insecticides.

II. *Determination of the Specific Rotation of B1 and B2*

The specific rotation value was the only different feature be observed between 2 optical isomers. The specific rotation $[\alpha]^{20}_{365}$ of 2 bifenthrin isomers, B1 and B2, was -66.41° and +69.63°, respectively. Therefore, we concluded that B1 was (-)-bifenthrin and B2 was (+)-bifenthrin. Accordingly, the specific rotation of isomers could be regarded as evidence proving that the separated compounds were different.

III. *Mass Spectrum*

The separated isomers were analyzed by GC-MS for further confirmation (Figure 2). Comparison of the retention times of separated isomers, (-)-bifenthrin, (+)-bifenthrin, and the standard bifenthrin showed that the observed retention times of these three compounds coincided with each other at 13.961 (±0.0068) min. These 3 compounds showed the same major fragments including *m/z* 115, *m/z* 141, *m/z* 165, *m/z* 181, and *m/z* 422.

IV. *Biological Toxicity Test*

Understanding the effects of separated isomers on aquatic organisms is an important contribution to environmental hazard assessment. The toxicity of separated isomers (-)-bifenthrin or (+)-bifenthrin on 3 kinds of aquatic fauna, daphnia, carp and tilapia was evaluated. According to the data shown in Table 3, after 12 hr of exposure, the LC_{50} of (-)-bifenthrin and (+)-bifenthrin for *Daphnia pulex* was 2.1 μg/L and 28.9 μg/L, respectively. For *Cyprinus carpio*, the LC_{50} (96 hr) for (-)-bifenthrin and (+)-bifenthrin was 0.99 μg/L and 2.08 μg/L, respectively. For *Tilapia* spp., the LC_{50} (96 hr) was 0.19 μg/L for (-)-bifenthrin and 0.80 μg/L for (+)-bifenthrin. As a result, the toxicity of (-)-bifenthrin was 10-fold greater than (+)-bifenthrin based on the acute toxicity test for daphnia at 12 hr. Moreover, the fish toxicity test (96 hr) indicated the toxicity of (-)-bifenthrin was 2-fold greater than (+)-bifenthrin.

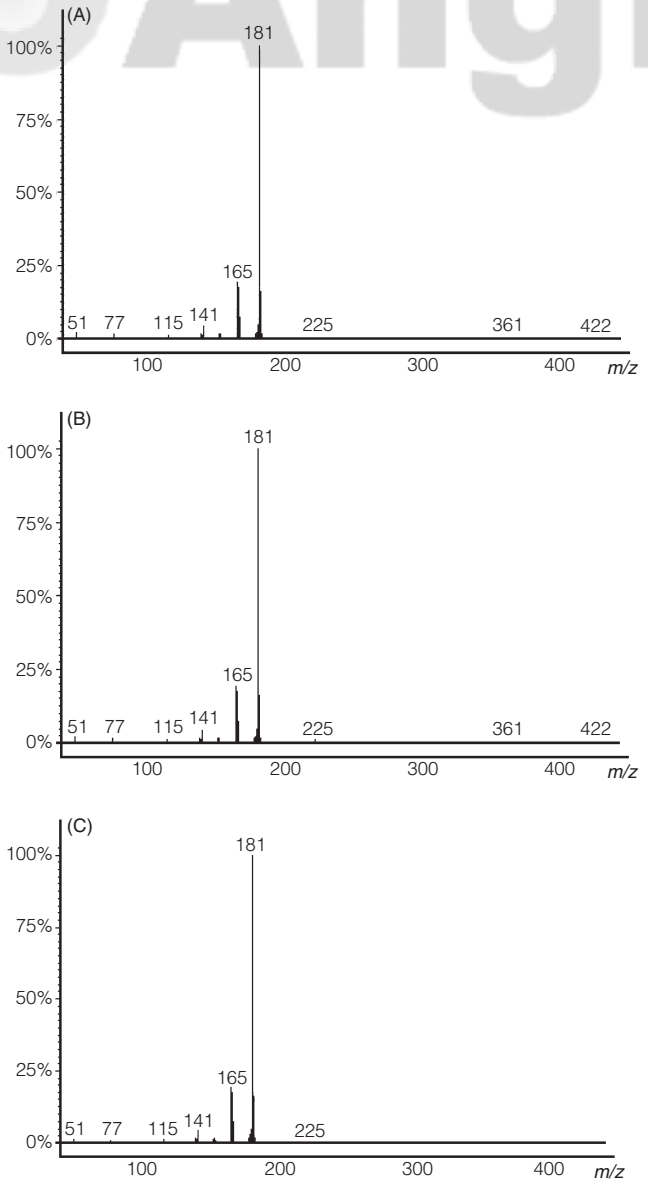


Figure 2. Mass spectra of bifenthrin (A) and separated enantiomers (-)-bifenthrin (B), (+)-bifenthrin (C).

CONCLUSIONS

The enantiomers of bifenthrin were separated successfully and efficiently by HPLC with a Sumichiral OA-2500-I chiral column. The solvent system composed of *n*-hexane, isopropanol, and ethanol in a ratio of 99.8/0.06/0.14 had better resolution in a reasonable elution time. The toxicity of (-)-bifenthrin was greater than (+)-bifenthrin for both daphnia and fish. This study provided a method of separating bifenthrin enantiomers by chiral HPLC and a toxicity test of individual enantiomers for non-target aquatic fauna.

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