Direct Chiral Resolution and its Application to the Determination of the Pesticide Tetramethrin in Soil by High-Performance Liquid Chromatography Using Polysaccharide-Type Chiral Stationary Phase

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Abstract

A high-performance liquid chromatography method is presented for the enantioseparation and quantitation of tetramethrin. The separation is achieved on amylose 3,5-dimethylphenyl-carbamate CSP (Chiralpak AD-H column) using a mobile phase consisting of a mixture of *n*-hexane–ethanol–2-propanol (99:0.9:0.1, v/v/v). Baseline chiral separation for the four isomers of tetramethrin is obtained within 20 min. Each of the resolutions of the two pairs of enantiomers is more than 2.0. The absolute configurations of each isomer are determined. The accuracy, precision, linearity, and limits of detection and quantitation of the method are investigated. For the determination of isomers in soil, the soil sample is extracted with acetone in an ultrasonic bath. The percentage recoveries from soil are in the range of 73.5% to 87.9%.

Introduction

Chiral pesticides have attracted great attention in recent years, and one-fourth of commonly used pesticides are chiral (1). Tetramethrin, which is a mixture of four stereoisomers was first synthesized in 1964 by Kato et al. (2). The (1R,*trans*) isomer is the most biologically active of the isomers, followed by the (1R,*cis*) isomer. The acute oral toxicity of tetramethrin is low. In acute inhalation studies, the LC_{50} in rats and mice was 2500 mg/m^{-3} for the racemic mixture and > 1180 mg/m^{-3} for the (1R, *cis*/*trans*) isomer (3). Because of the different effects of enantiomers on living organisms, it is necessary to develop new chiral separation methods to measure tetramethrin isomers in environmental samples.

Several methods have been described for the extraction and determination of tetramethrin pesticide residues in vegetables (4–6), milk (7), and soil (8,9). For the enantiomeric separation of tetramethrin, however, few reports have previously been published. As far as we know, the complete separation of all four enantiomers from tetramethrin has not been reported. The Supelcosil LC-CN precolumn and the Chiralcel OD-H column were chained to separate the *cis*-tetramethrin enantiomers (10). Liu and co-workers reported the chiral separation of tetramethrin by a capillary column containing β-cyclodextrin derivative stationary phase in gas chromatography, and obtained three peaks that were not completely separated at the baseline (11). Elution of tetramethrin enantiomers on Whelk-O CSPs resulted in two separated peaks (12).

In this paper, chiral resolution of the tetramethrin enantiomers was investigated on amylose 3,5-dimethylphenyl-carbamate CSP (Chiralpak AD-H). The quantitative analysis of the enantiomers of tetramethrin and residual analysis in soil samples were also performed in the study. The purpose of the work is to set up methods for quantitative and residual analysis of the tetramethrin enantiomers.

Experimental

Apparatus and reagents

The high-performance liquid chromatography (HPLC) system consisted of a Waters 515 pump, a 2487 dual λ absorbance detector (Milford, MA), and a Rheodyne Model 7725i injector (Cotati, CA). Chromatogram acquisition and processing was performed by Junrui SrAdv software package (China). The column temperature was regulated and controlled by an HCT-360 HPLC column thermostat (Quandao, Shanghai, China). The chiral column employed was Chiralpak AD-H column (150 mm × 4.6 mm i.d., Daicel Chemical Industries Ltd., Japan), which was packed with 5 µm silica-gel coated with amylose *tris* (3,5-

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dimethylphenyl carbamate). Also used were an ultrasonic bath (Branson Ultrasonics Corporation, China), a rotary evaporator (RE-52AA, Shanghai Yarong Instrument Co., China), and an advanced laser polarimeter (PDR-Chiral Inc., Lake Park, FL).

Analytical-grade *n*-hexane, 2-propanol, and ethanol were obtained from Shanghai Reagent Company (Shanghai, China.) Pure distilled water was purchased from Shanghai Jiazhou Sparkling Sources Co., Ltd. (Shanghai, China). *Trans*-enriched tetramethrin (Figure 1) was from China Pharmaceutical University (Nanjing, China). Pesticide solutions were prepared by dissolving the samples in *n*-hexane–2-propanol (70/30, v/v) at a concentration of 1.0 mg/mL and injected after filtration on a 0.45 µm Millipore filter (Milford, MA). Tetramethrin stock solution was prepared in concentration of 503.1 µg/mL.

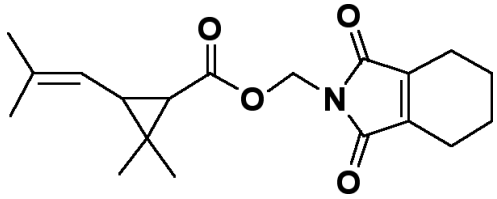


Figure 1. Chemical structure of tetramethrin.

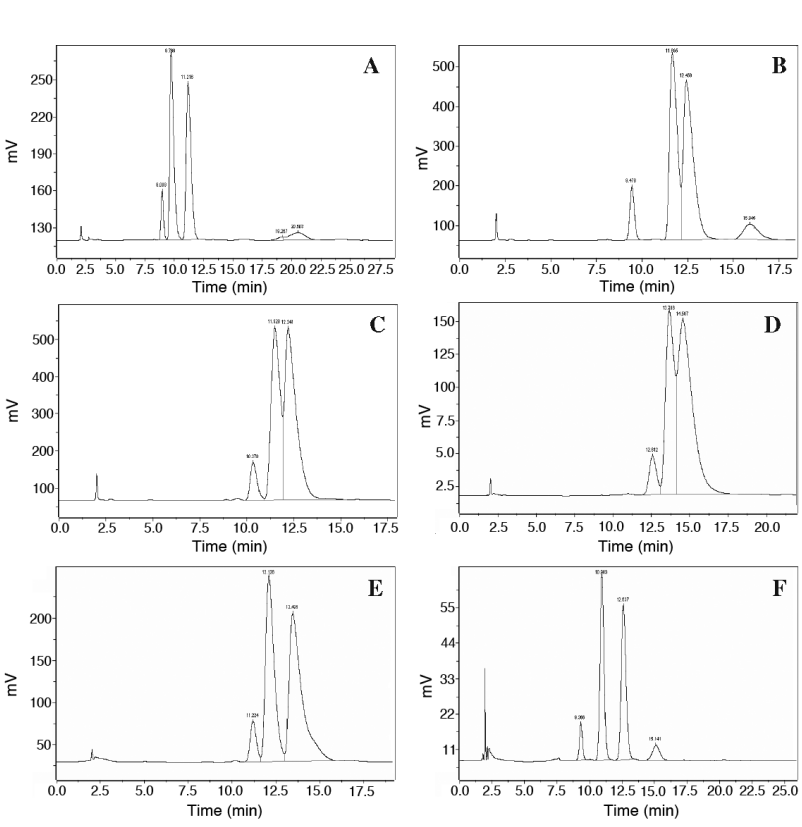


Figure 2. The effect of modifiers at room temperature on the enantioseparation of tetramethrin. Chromatographic conditions: Binary-compounds mobile phases included *n*-hexane–methanol = 99:1(v/v) (A); *n*-hexane–ethanol = 99:1(v/v) (B); *n*-hexane–2-propanol = 99:1(v/v) (C). Triad-components mobile phases included *n*-hexane–ethanol–2-propanol = 99:0.2:0.8 (v/v/v) (D); *n*-hexane–ethanol–2-propanol = 99:0.5:0.5 (v/v/v) (E); *n*-hexane–ethanol–2-propanol = 99:0.9:0.1 (v/v/v) (F); room temperature; 1.0 mL/min; 214 nm.

Chromatographic conditions

The mobile phase was *n*-hexane–ethanol– isopropanol used at a flow rate of 1.0 mL/min. The column was maintained at 35°C with detection via UV at 214 nm. The injection volume was 20 µL. The dead-time (t_0) of the column was determined by injecting 5 µL of a 1.0 mg/mL solution of tri-*tert*-butylbenzene (Acros, Geel, Belgium).

Preparation of fortified soil samples

Blank soil was collected from the garden of Shanghai Institute of Organic Chemistry. The material was dried at room temperature in air for 48 h. Dried material was powdered by a mortar and then sieved (60 mesh). To prepare tetramethrin-spiked soil samples, 10.0 mL of proper solution of tetramethrin (at the concentrations of 5.34 µg/mL, 27.1 µg/mL, and 104.4 µg/mL, respectively) were added to 10 g of blank soil in 250-mL conical flasks, which resulted in quality control samples of 5.34 mg/kg, 27.1 mg/kg, and 104.4 mg/kg, respectively. After the addition of 5 mL pure distilled water, and shaking for 1 min, the soil sample was allowed to equilibrate for 90 min prior to extraction. The sample was then extracted with 25 mL of acetone in ultrasonic bath for 15 min. The mixture was filtered and the residual soil was also extracted by acetone (15 mL × 2). The extracted solutions were combined and concentrated to dryness by removing the acetone solvent in a rotary evaporator at 35ºC, and evaporated to dryness at 30ºC under a gentle stream of nitrogen, and the residue was reconstituted in 10 mL *n*-hexane–2-isopropanol (70/30, v/v).

Results and Discussion

Chiral separation on Chiralpak AD-H

Tetramethrin was separated using polysaccharide-type CSP in an *n*-hexane–alcohol mobile phase. Optimization of the chromatographic conditions was done, including investigating the effect of the type of alcohol modifier and column temperature on the resolutions.

Among several alcohols used, the best separation was obtained using methanol. Unfortunately, as methanol is not miscible in *n*-hexane, mixing them caused an instable system. It was difficult to use a binary mixture *n*-hexane–alcohol mobile phase for a complete separation. Then, triad-component mobile phases were investigated. The addition of 2-propanol in *n*-hexane–ethanol improved the separation of tetramethrin. As shown in Figure 2F, the best result was achieved when 0.9% ethanol and 0.1% 2-propanol were added simultaneously.

The effect of temperature was also studied. For *trans*-tetramethrin racemates, selectivities (α) were approximately constant when temperature was increased from 10°C to 35°C (1.152 and 1.179

at 10°C and 35°C, respectively), while the enantioselectivity for *cis* racemates dramatically improved with increasing temperature (1.633 and 1.760 at 10°C and 35°C, respectively). Under such circumstances, the enantioseparation was carried out at a higher investigated temperature. The complete separation of the four stereoisomers was achieved at 35ºC.

Elution orders

Combining the chiral HPLC and polarimeter detector is an important technique for identifying the elution orders of enantiomers. The polarity (i.e., rotation sign) was indicated directly from the appearance of a positive (+) or negative (–) peak on the polarimeter concurrent to the response on the UV detector. Because the tetramethrin used is *trans*-enriched, the isomer E2 and E3 with large peak area are all trans isomers. According to a previous reference (13), 1R isomers are positive (+) peak on the polarimeter. Then the absolute configuration of tetramethrin could be determined, and Table I shows the results.

Table I. Elution Orders of Tetramethrin on Chiralpak AD-H

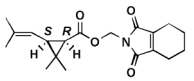
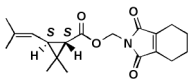
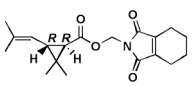
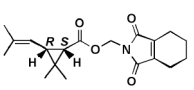
Isomer	Polarity	Name	Structure
E1	+	(1R)-*cis*-tetramethrin	
E2	–	(1S)-*trans*-tetramethrin	
E3	+	(1R)-*trans*-tetramethrin	
E4	–	(1S)-*cis*-tetramethrin	

Table II. Validation of the Quantitative Analysis Method for the Enantiomers*,†

Isomer	Linear equation	*r*	LOD (µg/mL)	LOQ (µg/mL)
E1	$Y = 59088x + 2478.34$ (Linearity range 0.40–31.62 µg/mL)	0.9999	0.06	0.20
E2	$Y = 57653x + 17512.46$ (Linearity range 1.13–220.86 µg/mL)	0.9999	0.08	0.26
E3	$Y = 57874x + 14877.66$ (Linearity range 1.13–220.12 µg/mL)	0.9999	0.09	0.30
E4	$Y = 62181x + 2397.57$ (Linearity range 0.98–30.49 µg/mL)	0.9999	0.16	0.53

* E1, E2, E3, and E4 represent the first, the second, the third, and the last eluted isomers, respectively.
† *Y* corresponds to the peak areas and *x* refers to the each tetramethrin enantiomer concentration expressed in µg/mL.

Linearity and sensitivity

Calibration curves for the first three tetramethrin enantiomers were linear as shown in Table II. The correlation coefficients (*r*) were all greater than 0.9999.

Precision and accuracy

Intra-day precisions of the method were evaluated by assaying freshly prepared solutions at a concentration of 40.25 µg/mL of tetramethrin. To evaluate the inter-day reproducibility of the method, three solutions of different total tetramethrin concentrations of 4.66, 27.62, and 55.24 µg/mL were tested on three consecutive days. Table III shows intra- and inter-day precision (% RSD). The repeatability at the levels represented in Table III is satisfactory, with an RSD below 5% in all cases.

Recovery studies were carried out with 10 g of blank soil spiked with 10 mL proper tetramethrin solutions at three concentration levels to make final concentrations as 5.34, 27.1, and 104.4 mg/kg in the blank soil, respectively. Figure 3 presents the chromatograms of spiked soil samples. The absolute recovery of tetramethrin enantiomers at three concentration levels was determined by comparing the peak areas measured after the analysis of spiked soil samples with standard sample solutions at the same concentration levels. The recoveries of pyrethroid isomers from soil varied from 73.5% to 87.9% at three concentration levels, with an RSD below 10%.

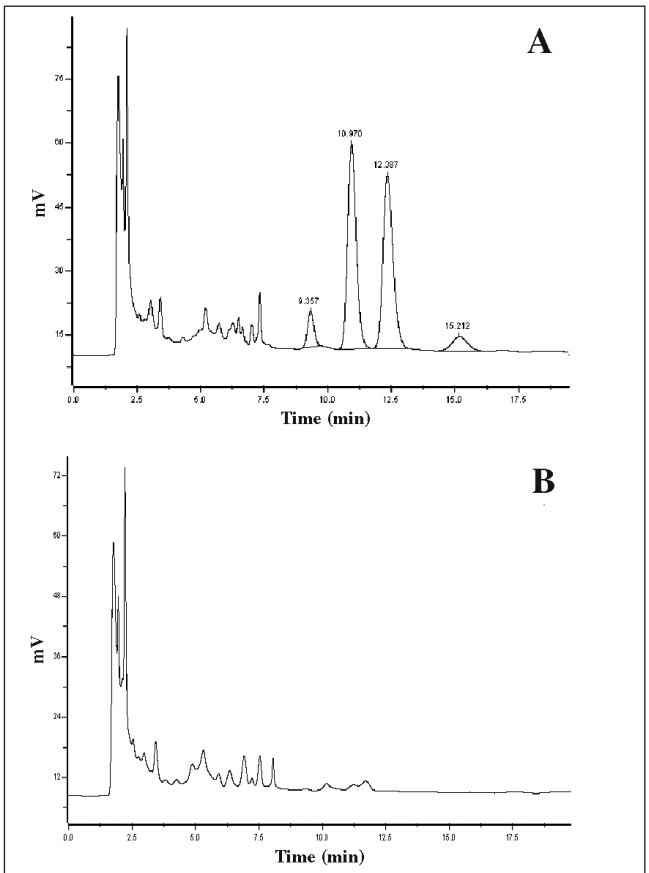


Figure 3. Chromatograms of samples extracted from spiked 104.4 (A) and 2.57 (B) mg/kg tetramethrin soil. Chromatographic conditions: Mobile phase: *n*-hexane–ethanol–2-propanol = 99/0.9/0.1(v/v/v), 1.0 mL/min; 214 nm, 35°C.

Table III. Repeatability Results of the Chiral HPLC Method

Validation parameter		Inter-day peak area results			(% RSD)
Isomer	Concentration (µg/mL)	Day 1 (n = 3)	Day 2 (n = 3)	Day 3 (n = 3)	Average (n = 9)
E1	4.66	1.96	1.07	3.25	2.84
	27.62	2.86	2.86	0.85	3.34
	55.24	0.81	0.30	0.97	0.73
E2	4.66	1.03	1.08	0.14	0.91
	27.62	2.21	0.97	1.43	1.58
	55.24	0.63	1.88	1.92	1.40
E3	4.66	2.51	0.87	0.15	1.63
	27.62	2.89	1.02	0.32	1.69
	55.24	0.36	1.38	1.51	1.05
E4	4.66	3.26	2.16	3.56	2.66
	27.62	1.66	1.30	0.52	1.55
	55.24	1.51	0.92	1.77	1.50
Intra-day results (40.25 µg/mL, n = 6, % RSD)					
		E1	**E2**	**E3**	**E4**
Retention time		0.09	0.03	0.47	0.06
Peak area		0.59	0.36	0.44	1.48

Conclusions

A new and accurate normal phase chiral HPLC method was described for the enantiomeric separation of tetramethrin on Chiralpak AD-H. Four baseline-separated peaks were obtained for tetramethrin. The elution sequence and absolute configurations were determined. The method was also investigated, including linearity, precision, limit of detection, and limit of quantitation. The extraction procedure and the chromatographic setup are simple and reliable. The developed chiral separation method may be used to evaluate enantioselectivity of tetramethrin during biodegradation, toxicity, and other environmental processes.

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