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Separation and Analysis of Diastereomers and Enantiomers of Cypermethrin and Cyfluthrin by Gas Chromatography

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Synthetic pyrethroid (SP) insecticides are of environmental significance because of their high aquatic toxicity. Due to their chirality, SP compounds contain multiple diastereomers and enantiomers. However, due to great structural similarities and lack of isomer standards, gas chromatographic (GC) analysis of SP diastereomers or enantiomers is poorly developed. In this study, we used a HP-5 column to separate the diastereomers and a β-cyclodextrin-based enantioselective column (BGB-172) to separate the enantiomers of cypermethrin (CP) and cyfluthrin (CF). Resolved peaks were identified by comparing chromatograms of isomer-enriched CP products. Diastereomers of both CP and CF were separated on the HP-5 column. On the BGB-172 column, enantiomers of all cis diastereomers were separated, while those of trans diastereomers were not separated. The elution order appears to be regulated by configuration, a finding which may allow peak identification in the absence of isomer standards. When coupled with electron capture detection, the developed methods had low detection limits and may be used for analysis of SP diastereomers and enantiomers in environmental samples.

KEYWORDS: Chiral analysis; diastereomer separation; enantiomer separation; synthetic pyrethroids; enantioselectivity

INTRODUCTION

Many pesticides and other organic compounds are chiral compounds containing stereoisomers. Pairs of enantiomers, or diastereomers, usually differ significantly in both physicochemical properties and biological activity (*1*). Enantiomers from the same diastereomer have the same physicochemical properties (*1*, *2*) but likely different biological properties (*3*). In the environment, enantiomers may be selectively degraded by microorganisms (*3*−*5*). Such enantioselectivity has been shown to result in different distribution patterns (*6*−*8*), and bioaccumulation potentials between enantiomers in the environment (*9*, *10*).

Synthetic pyrethroid (SP) insecticides contain 2 or 3 stereogenic centers, making them a pesticide group with one of the highest chirality. Thus, each SP compound contains 2 or 4 enantiomer pairs, or 2 or 4 diastereomers. SPs are widely used on crops, on animals, and in households. Their use may further increase as the use of organophosphate insecticides and carbamate becomes increasingly restricted. SPs are of environmental concern because of their high acute toxicity to fish and aquatic invertebrates (*11*). For instance, the LC50 values of cypermethrin (CP) and cyfluthrin (CF) to *Daphnia magna* are 2 μg L^{-1} and 0.2 μg L^{-1}, respectively (*12*, *13*). It is known that only some stereoisomers of a SP compound possess biological activity (*14*−*16*). For instance, the activity of CF against the aquatic invertebrate *Daphnia magna* and various strains of *Lepidoptera* was found to derive mainly from only two (*1R-3R*-α*S* and *1R-3S*-α*S*) of the eight possible stereoisomers (*16*). Moreover, studies using ^{14}C-labeled isomers showed that the rate of soil degradation was isomer-specific for CP (*17*). Significantly faster degradation occurred with the trans diastereomers than with the corresponding cis diastereomers, and in the same diastereomers, with the αS-enantiomer than with the corresponding αR enantiomer. However, isomeric selectivity has been generally ignored in environmental analysis of SP residues, which may be due to the great difficulty in separating and identifying SP stereoisomers. The difficulty is manifested by a general lack of standards that are hard to synthesize or purify. In previous studies, enantioselective analysis of SPs was confined mostly to HPLC (*18*−*20*), and the published GC methods were only capable of separating SP diastereomers (*21*−*23*). Analysis by HPLC, however, suffers the drawback of low sensitivity, making it unsuitable for environmental analysis.

In this study, we used cypermethrin ((*RS*)-α-cyano-3-phenoxybenzyl (*1RS*)-*cis-trans*- 3-(2,2-dichlorovinyl)-1,1-dimethylcyclopropanecarboxylate) and cyfluthrin ((*RS*)-α-cyano- 4-fluro-3-phenoxybenzyl (*1RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-1,1-dimethylcyclopropane- carboxylate) as model SP compounds and evaluated their resolution on achiral and enantioselective GC columns. The elution orders of diastereomers and enantiomers were determined by comparing chromatograms of isomer-enriched products. The developed methods were further applied to analysis of water and sediment samples.

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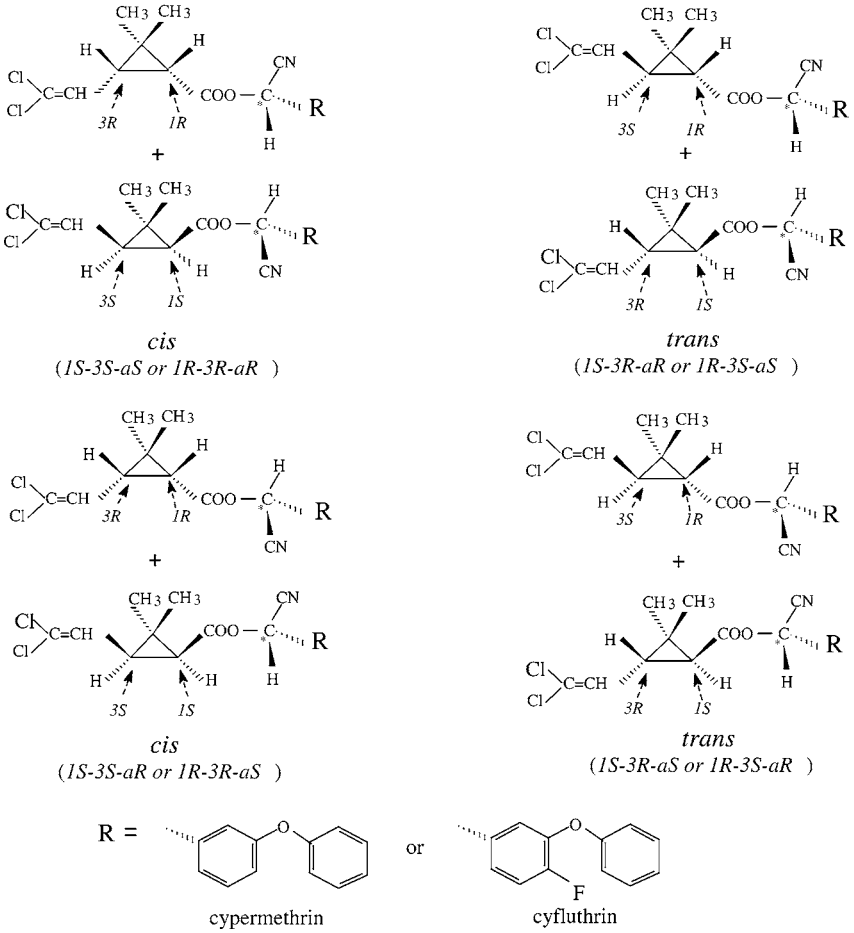

Figure 1. Structures of cypermethrin or cyfluthrin enantiomers as diastereomers (enantiomer pairs).

MATERIALS AND METHODS

Structural Description. The SPs used in this study are structurally similar, with the only difference being the substitution of F in the phenoxybenzyl structure in CF (**Figure 1**). Both CP and CF contain two asymmetric carbons in the cyclopropyl ring, and one asymmetric carbon at the α-cyano carbon. Therefore, nonenriched CP or CF consists of eight stereoisomers, which form four enantiomer pairs or diastereomers: *1S-3S*-α*S* + *1R-3R*-α*R* (cis), *1R-3S*-α*R* + *1S-3R*-α*S* (trans), *1R-3R*-α*S* + *1S-3S*-α*R* (cis), and *1S-3R*-α*R* + *1R-3S*-α*S* (trans) (**Figure 1**). In CP and CF, *1R-3R*-α*S* and *1R-3S*-α*S* possess much greater insecticidal activity than the other stereoisomers (*14*, *16*). The active stereoisomers are intentionally enriched in α-CP, *β*-CP, and *θ*-CP to achieve greater insecticidal efficacy.

Chemicals. Nonenriched CP (CP, 98%), nonenriched CF (CF, 98%), and *β*-CP (86%, enriched in *1R-3R*-α*S* + *1S-3S*-α*R* and *1R-3S*-α*S* + *1S-3R*-α*R*) were purchased from Chem Service (West Chester, PA). The isomer-enriched formulations α-CP (99%, enriched in *1S-3S*-α*S* + *1R-3R*-α*R* and *1R-3R*-α*S* + *1S-3S*-α*R*) and *θ*-CP (99%, enriched in *1R-3R*-α*S*) were provided by FMC (Princeton, NJ). Stock solutions were prepared by dissolving the SP standards in acetone at 1.0 mg ml^{-1} and stored at 4 °C. Working solutions were prepared daily by diluting the stock solutions with acetone-hexane (1:1, v/v). Other chemicals used in this study were of pesticide residue or analytical-reagent grade.

Chromatographic Separation and Analysis. An Agilent 6890N GC system with electron capture detector (ECD) and 5973N mass selective detector (MSD) were used for the analysis. The temperature of the ECD was 310 °C, and the detector makeup gas was N_2 (60 mL min^{-1}). The MSD was operated in the electron-impact ionization mode (EI, 70 eV, 230 °C) and full scan mode (*m*/*z* 40−445, 1.2 s $scan^{-1}$). The inlet temperature was 260 °C, and 1.0 μL was introduced in the splitless mode. The flow rate of the carrier gas (helium) was 1.0 mL min^{-1}.

Primary analysis was conducted using GC-ECD, and confirmatory analysis was carried out using GC-MSD. Achiral GC analysis for separating diastereomers was carried out by using a 30-m × 0.25-mm × 0.25-μm HP-5 column (cross-linked 5% diphenyl and 95% dimethylpolysiloxane, Agilent, Wilmington, DE). The initial column temperature was 180 °C for 2 min, and ramped at 5 °C min^{-1} to 280 °C, followed by an isothermal hold at 280 °C until complete elution. In preliminary experiments, a number of chiral capillary columns were tested for their capability to achieve enantioselective resolution of the test compounds. These columns included *β*-DBX (30-m × 0.25-mm × 0.25-μm, 20% permethylated *β*-cyclodextrin in 35% diphenyl- and 65% dimethylsiloxane, Supelco, Bellefonte, PA), Agilent Cyclosil-B (30-m × 0.2-mm × 0.25-μm, 30% hepatkis (2,3-di-*O*-methyl-6-*O*-butyl dimethylsilyl)-*β*-cyclodextrin in DB 1701, Agilent), BGB-176 (30-m × 0.25-mm × 0.25-μm, 20% 2,3-dimethyl-6-*tert*- butyldimethylsilyl-*β*-cyclodextrin in 15% diphenyl- and 85% dimethyl-polysiloxane, Analytik, Adliswil, Switzerland), and BGB-172 (30-m × 0.25-mm × 0.25-μm, 20% *tert*-butyldimethylsilyl-*β*-cyclodextrin in 15% diphenyl- and 85% dimethylpolysiloxane, BGB Analytik). The best resolution was obtained with the BGB-172 column, which was used for further method development in this study. In the final analytical protocol, the column was initially held at 160 °C for 2 min, ramped at 1 °C min^{-1} to 220 °C (first ramp), held at 220 °C for 60 min, ramped at 5 °C min^{-1} to 230 °C (second ramp), and held at 230 °C until complete elution. Retention times were used as the sole criterion for identifying peaks in GC-ECD chromatograms. In the GC-MSD analysis, distribution patterns of ion fragments, molecular ions, and chloride isotope peaks were used as the key criteria for identification.

Previous studies showed that pyrethroids may undergo epimerization under certain conditions (*16*). To evaluate the stability of diastereomers and enantiomers during analysis, racemic CF was resolved on the BGB-172 column under the same conditions given above while varying the inlet temperature from 200 to 260 °C. Isomer stability was further

evaluated by analyzing water samples spiked with racemic CF at 100 μg L^{-1} following incubation at room temperature up to 5 days. Procedures used for sample preparation were the same as described below. Relative peak areas were computed for the resolved peaks and used for evaluating isomer stability.

Method Evaluation. The developed methods were applied to water and sediment samples to evaluate their applicability for analysis of environmental samples. To analyze water samples, 40 mL of deionized water was spiked with stock solutions to give increasingly lower SP concentrations, ranging from 150 to 0.5 μg l^{-1}. The spiked water samples were then extracted twice by mixing with 30 mL of ethyl acetate in a 250-mL separatory funnel. The ethyl acetate phase was collected and concentrated to near dryness on a rotary evaporator at 60 °C. The final residues were recovered in 4.0 mL acetone/hexane (1:1, v/v) and used for GC analysis. For sediment analysis, sediment samples (containing 1.11% organic carbon, 87% sand, 8% silt, and 5% clay) in glass vials were spiked with racemic CP or CF at 100 μg kg^{-1} and incubated at room temperature. Samples were periodically removed and extracted with hexane/acetone (1:1, v/v) by mixing and centrifugation. Extracts from three consecutive extractions were combined, passed through a layer of anhydrous sodium sulfate, and then concentrated to dryness on a rotary evaporator at 60 °C. The final residues were recovered in 4.0 mL acetone/hexane (1:1, v/v) and used for achiral and enantioselective GC analysis. Quantitative data of CP and CF isomers were obtained by the external calibration method using standards, assuming the same response factor for isomers with the same molecular structure.

RESULTS AND DISCUSSION

Separation of Diastereomers. The different physicochemical properties of SP diastereomers allowed their adequate separation on the achiral HP-5 column. Under the tested conditions, nonenriched CP and CF both produced four peaks that were resolved at the baseline. The retention times of the four resolved peaks from CP were 35.0 (I), 35.3 (II), 35.5 (III), and 35.7 (IV) min, respectively (**Figure 2a**). The retention times of the four resolved peaks from CF were 32.8 (I), 33.1 (II), 33.3 (III), and 33.5 (IV), (**Figure 3a**). Standards of α-CP and θ-CP both gave two peaks with identical retention times of 35.0 (I) and 35.5 (III) min, with III being the predominant peak (**Figure 2**, parts **b** and **d**). The appearance of peak I in the θ-CP chromatogram suggests the presence of a minor impurity (17.8%). Analysis of β-CP gave four peaks (I−IV) with retention times identical to those of CP (**Figure 2**, parts **b** and **d**). The β-CP formulation therefore also contained impurities, and the peak area increased in the order I (5.7%) < II (9.7%) < III (30.7%) < IV (53.9%).

The use of various isomer-enriched CP formulations allowed the assigning of specific diastereomers to the resolved peaks. From the chromatogram of θ-CP (enriched in *1R-3R-*α*S*), peak III was identified as the cis diastereomer *1R-3R-*α*S* +*1S-3S-*α*R* (**Figure 2d**). The difference between the chromatogram of α-CP (enriched in *1S-3S-*α*S* + *1R-3R-*α*R* and *1R-3R-*α*S* + *1S-3S-*α*R*) and that of θ-CP suggests that peak I belonged to the other cis diastereomer (*1S-3S-*α*S* and *1R-3R-*α*R*) (**Figure 2**, parts **b** and **d**). The dominant composition of peaks III and IV in the chromatogram of β-CP (enriched in *1R-3R-*α*S* +*1S-3S-*α*R* and *1R-3S-*α*S* + *1S-3R-*α*R*) suggests that peak IV was the second trans diastereomer (*1R-3S-*α*S* + *1S-3R-*α*R*), leaving peak II as the first trans diastereomer (*1R-3S-*α*R* + *1S-3R-*α*S*).

Similar elution orders were observed in previous studies for CP and CF by other researchers. Leicht et al. (*16*) separated diastereomers of CF on a HP-1 capillary column and obtained four peaks eluted in the same order as observed in this study, although no standards were used to validate the peak assignment. Four peaks were also detected by Bolygo and Hadfield (*21*) and Hadfield et al. (*22*) for CP using capillary columns, and

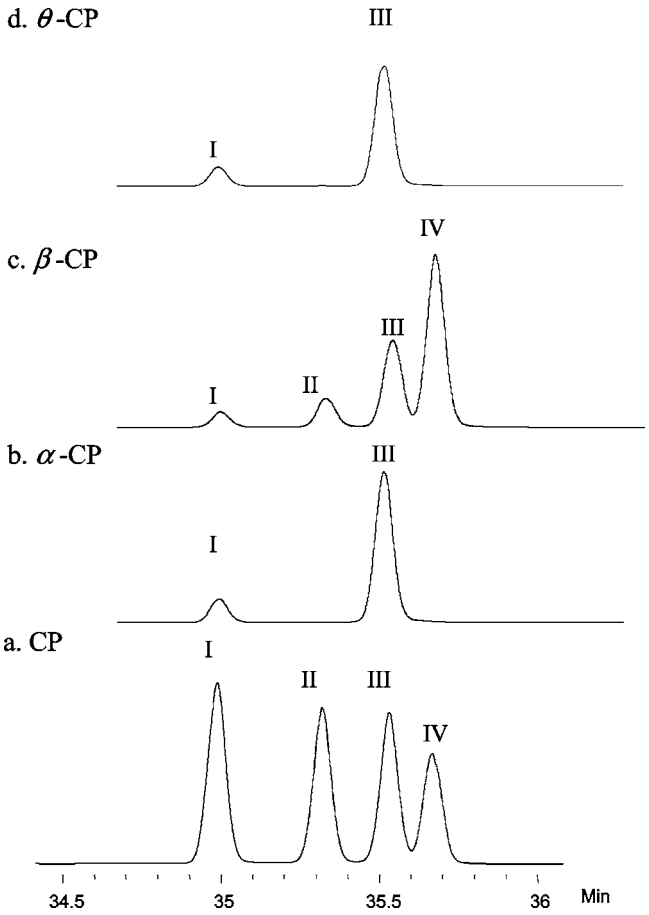

Figure 2. Achiral GC chromatograms of cypermethrin. (**a**) Mixture of racemic diastereomers of cypermethrin; (**b**) α-cypermethrin (enriched in *1S-3S-*α*S* + *1R-3R-*α*R* and *1R-3R-*α*S* + *1S-3S-*α*R*); (**c**) β-cypermethrin (enriched in *1R-3R-*α*S* + *1S-3S-*α*R* and *1R-3S-*α*S* + *1S-3R-*α*R*); and (**d**) θ-cypermethrin (enriched in *1R-3R-*α*S*).

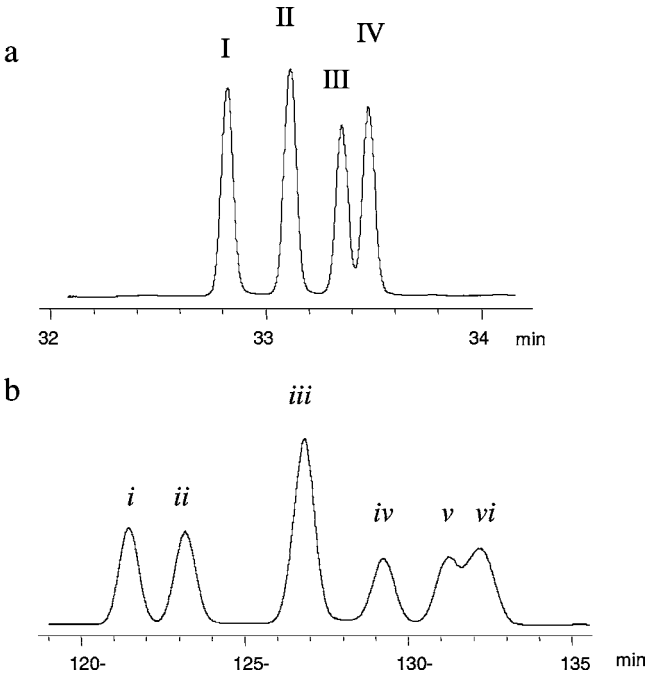

Figure 3. Achiral (**a**) and chiral (**b**) GC chromatograms of cyfluthrin.

the resolved peaks were assigned with diastereomers in the same order as in this study. Using analytical standards of individual diastereomers, Jin and Webster (*23*) also assigned the same order

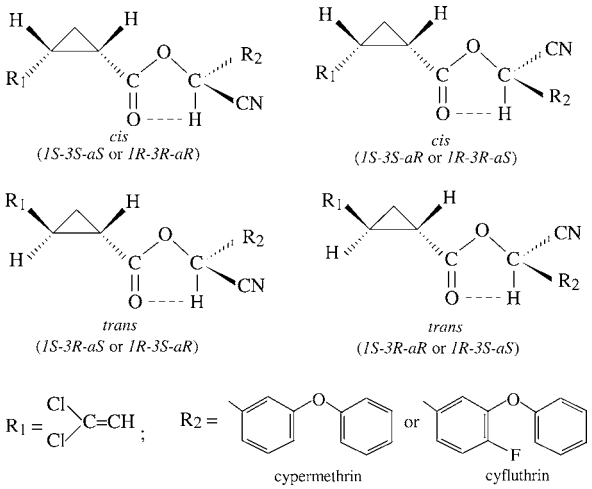

Figure 4. Configurations of cypermethrin or cyfluthrin showing potential hydrogen bond formation at the α-cyano carbon position.

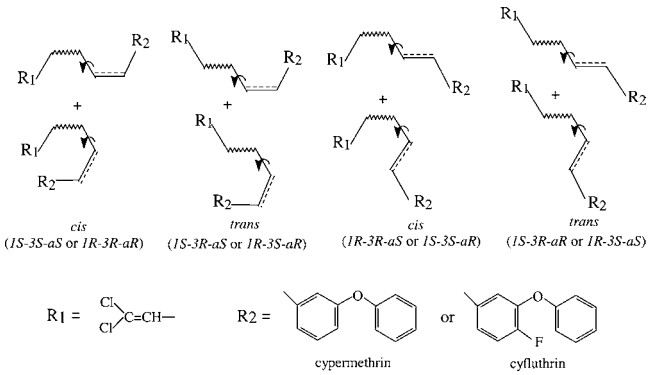

Figure 5. Configurations of cypermethrin or cyfluthrin as outlines, where double lines represent ring structures to show structural rigidity and arrows represent points that may rotate.

of diastereomers to the four resolved peaks on a DB-5 column that closely resembled the HP-5 column used in this study.

No previous effort was made to explain the specific elution order of CP or CF diastereomers on nonpolar GC columns such as the HP-1 and HP-5 columns. Although it is known that vapor pressure generally controlled the elution order of nonpolar compounds on nonpolar columns, vapor pressures of individual SP diastereomers are not available. However, the specific elution order of diastereomers of CP or CF may be tentatively explained from their configurations. It is likely that H on the α-cyano carbon may form a hydrogen bond with the carbonyl oxygen, producing a five-member inner ring that would decrease the rotation ability of the α-cyano carbon (**Figure 4**). The potential layouts of all CP or CF diastereomers are depicted in **Figure 5** as outlines, in which the rings are abbreviated as double lines to show the structural rigidity. With this assumption, the structural linearity increases in the order of (*1S-3R-*α*R* + *1R-3S-*α*S*) > (*1R-3R-*α*S* + *1S-3S-*α*R*) > (*1S-3R-*α*S* + *1R-3S-*α*R*) > (*1R-3R-*α*R* + *1S-3S-*α*S*) (**Figure 5**). According to the general rule of organic chemistry, the stability should increase, or vapor pressure should decrease, with increasing linearity for the same structure, which would suggest that the elution order of CP or CF diastereomers should follow the order given in **Figure 5**, with the retention time increasing from the left to the right.

Separation of Enantiomers. Several studies have reported that enantioselective columns coated with β-cyclodextrin derivatives provided adequate enantiomer separation for some chiral compounds (*4*, *5*, *24*−*26*). In this study, analysis of nonenriched CF or CP on the enantioselective BGB-172 column gave six

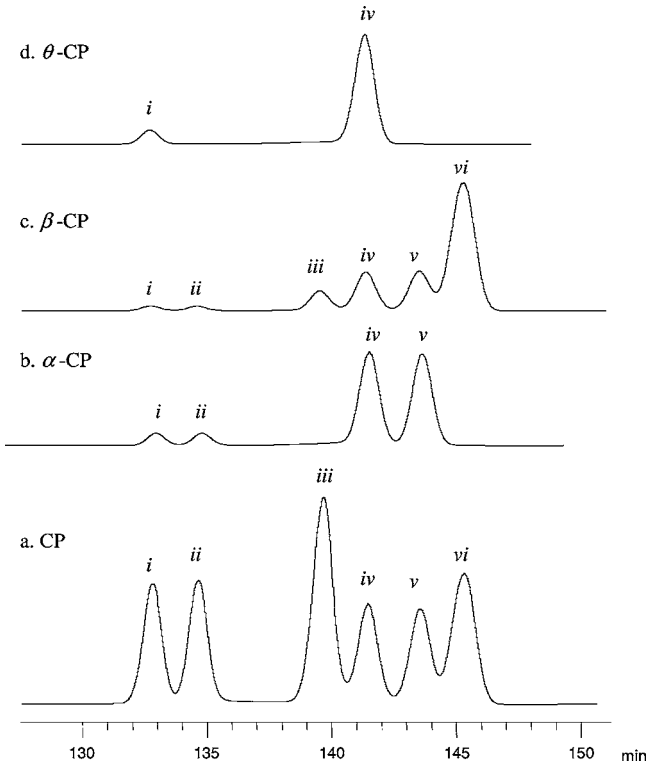

Figure 6. Chiral GC chromatograms of cypermethrin. (**a**) Racemic mixture of cypermethrin; (**b**) α-cypermethrin (enriched in *1S-3S-*α*S* + *1R-3R-*α*R* and *1R-3R-*α*S* + *1S-3S-*α*R*); (**c**) β-cypermethrin (enriched in *1R-3R-*α*S* + *1S-3S-*α*R* and *1R-3S-*α*S* + *1S-3R-*α*R*); and (**d**) θ-cypermethrin (enriched in *1R-3R-*α*S*).

Table 1. Retention Times and Peak Assignment of Diastereomers and Enantiomers of Cypermethrin and Cyfluthrin on Achiral (HP-5MS) and Enantioselective (BGB-172) GC Capillary Columns

	configuration				retention time (min)			
pesticide	*1C*	*3C*	α*C*	*1C*/*3C*	HP-5MS	peak	BGB-172	peak
cypermethrin	*R*	*R*	*R*	cis	35.0	I	132.6	*i*
	S	*S*	*S*	cis	35.0		134.5	*ii*
	R	*S*	*R*	trans	35.3	II	139.4	*iii*
	S	*R*	*S*	trans	35.3			
	R	*R*	*S*	cis	35.5	III	141.5	*iv*
	S	*S*	*R*	cis	35.5		143.6	*v*
	R	*S*	*S*	trans	35.7	IV	145.4	*vi*
	S	*R*	*R*	trans	35.7			
cyfluthrin	*R*	*R*	*R*	cis	32.8	I	121.3	*i*
	S	*S*	*S*	cis	32.8		123.2	*ii*
	R	*S*	*R*	trans	33.1	II	126.9	*iii*
	S	*R*	*S*	trans	33.1		126.9	
	R	*R*	*S*	cis	33.3	III	129.2	*iv*
	S	*S*	*R*	cis	33.3		131.2	*v*
	R	*S*	*S*	trans	33.5	IV	132.3	*vi*
	S	*R*	*R*	trans	33.5		132.3	

peaks (**Figures 6a** and **3b**). Analysis of α-CP resulted in four peaks, while that of θ-CP gave two peaks (**Figure 6**, parts **b** and **d**). Due to its poor purity, β-CP produced all six peaks (**Figure 6c**), but the relative peak areas were different from those of CP (**Figure 6a**).

Although enantiomer standards were not used, again it was possible to tentatively identify the resolved peaks on the enantioselective column by comparing the chromatograms between the enantiomer-enriched CP products (**Table 1**). From the specified composition of θ-CP (enriched in *1R-3R-*α*S*), peak *iv* (82.2%) was assigned as *1R-3R-*α*S* (**Figure 6d**). The assignment was consistent with the closeness in peak area

Table 2. Relative Peak Areas (% of Total Area) of Resolved Peaks of Cypermethrin and Cyfluthrin after Achiral and Chiral Separation and Detection by GC-ECD (Roman Numerals in Parentheses Correspond to Peaks Specified in Figures 2, 3, and 6)

	Achiral (HP-5)			
	I	II	III	IV
cypermethrin	30.4	25.6	24.9	19.1
α-cypermethrin	14.6	0	85.4	0
β-cypermethrin	5.7	9.7	30.7	53.9
θ-cypermethrin	17.7	0	82.3	0
cyfluthrin	26.8	28.3	22.1	22.8

	Enantioselective (BGB-172)					
	i	*ii*	*iii*	*iv*	*v*	*vi*
cypermethrin	14.3	14.8	25.9	12.3	12.3	19.4
α-cypermethrin	7.1	7.6	0	43.3	43.9	0
β-cypermethrin	2.9	2.9	9.9	15.7	15.3	53.9
θ-cypermethrin	17.8	0	0	82.2	0	0
cyfluthrin	13.6	13.9	30.5	10.8	10.9	20.1

Table 3. Relative Peak Areas (%) of Cyfluthrin Enantiomers on a Chiral BGB-172 Column with Different Inlet Temperatures and Following Extraction from Water or Clay Suspension Samples

		relative peak area (%)					
treatment		*i*	*ii*	*iii*	*iv*	*v*	*vi*
inlet temp	200 deg C	11.5	11.8	31.8	10.6	9.95	24.3
	220 deg C	11.1	11.4	32.4	10.6	9.96	24.5
	240 deg C	11.1	11.4	32.6	10.6	10.0	24.6
	260 deg C	11.2	11.2	32.9	10.3	9.77	24.6
water	1 day	11.4	11.8	32.2	10.6	9.96	24.6
	2 day	11.2	11.2	33.2	10.6	10.0	24.4
	5 day	11.3	11.4	33.1	10.3	9.77	24.7

between peak *iv* (82.2%) from the BGB-172 column and peak III (82.3%) from the HP-5 column (**Table 2**). From the relative peak composition, it was evident that in α-CP (enriched in *1S-3S-*α*S* + *1R-3R-*α*R* and *1R-3R-*α*S* + *1S-3S-*α*R*), peaks *i* (7.1%) and *ii* (7.6%) on the BGB-172 column were derived from peak I (14.6%) on the HP-5 column, and peaks *iv* (43.3%) and *v* (43.9%) on the BGB-172 column were derived from peak III (85.4%) on the HP-5 column. The presence of peak *v* in α-CP and the absence of a similar peak in *θ*-CP on the BGB-172 column suggest that peak *v* in the chromatogram of α-CP was *1S-3S-*α*R* (**Table 2**). The earlier elution of *1R-3R-*α*S* (*iv*) in relation to *1S-3S-*α*R* (*v*) further suggests that for the same cis diastereomer, the α*S* enantiomer was eluted before the corresponding *R* enantiomer. Applying the same rule to the first cis diastereomer, peaks *i* and *ii* may be labeled as *1R-3R-*α*R* and *1S-3S-*α*S*, respectively. Comparing the achiral and chiral GC chromatograms (**Figures 2c** and **6c**) of *β*-CP (enriched in *1R-3R-*α*S* + *1S-3S-*α*R* and *1R-3S-*α*S* + *1S-3R-*α*R*), the dominance of peak *vi* (53.9%) confirmed that peak *vi* was derived from the trans diastereomer *1R-3S-*α*S* +*1S-3R-*α*R* or peak IV (53.9%) on the HP-5 column (**Table 2**).

Due to the great structural similarities between CF and CP, the same elution order found for CP should be valid also for CF (**Table 1**). Samples of CP and CF were also eluted under the same conditions on GC-MS. In both cases, the resolved peaks were found to invariably contain mass spectra representative of the intact CP or CF molecule. Therefore, under the experimental conditions, partial separation was achieved using the BGB-172 column for all the stereoisomers contained in CP or CF. On the *β*-cyclodextrin-based enantioselective column, enantiomers from all the cis diastereomers were well separated, whereas those from all the trans diastereomers were not separated.

In the above discussion, it was assumed that stereoisomers of CP or CF did not undergo isomerization as a result of exposure to heat in the GC inlet or solvents used for extraction. When the GC inlet temperature was varied from 200 to 260 °C, the relative peak areas of the six resolved peaks for CF remained essentially unchanged (**Table 3**). Suggesting that exposure to heat in the inlet zone within the given temperature range did not cause thermal isomerization. Analysis of water samples containing CF showed that the relative peak areas did

Table 4. Method Detection Limits (MDL, in μg L^{-1}) and Standard Deviation (SD, %) of Achiral and Chiral Analysis of Diastereomers and Enantiomers of Cypermethrin and Cyfluthrin in Water by GC-ECD

	configuration			
treatment	*SSS* + *RRR*	*RSR* + *SRS*	*RRS* + *SSR*	*RSS* + *SRR*
cypermethrin	0.30	0.30	0.30	0.30
cyfluthrin	0.15	0.15	0.15	0.15

	RRR	*SSS*	*RSR* + *SRS*	*RRS*	*SSR*	*RSS* + *SRR*
cypermethrin	7.5	7.5	8.0	10	10	15
cyfluthrin	3.5	3.5	4.0	5.6	5.6	7.5

Table 5. Concentrations (μg kg^{-1}) of Cypermethrin and Cyfluthrin Diastereomers and Enantiomers in Sediment Following Incubation at Room Temperature

	configuration			
treatment	*SSS* + *RRR*	*RSR* + *SRS*	*RRS* + *SSR*	*RSS* + *SRR*
cypermethrin: 0 d	29.6 ± 1.3	25.4 ± 1.3	21.0 ± 1.4	19.9 ± 1.4
60 d	9.4 ± 1.4	4.2 ± 1.2	8.2 ± 1.5	3.5 ± 1.3
cyfluthrin: 0 d	22.1 ± 1.2	25.4 ± 1.3	21.0 ± 1.4	29.9 ± 1.4
60 d	7.2 ± 1.4	4.2 ± 1.2	8.2 ± 1.5	3.5 ± 1.3

	RRR	*SSS*	*RSR*+*SRS*	*RRS*	*SSR*	*RSS*+*SRR*
cypermethrin: 0 d	14.9 ± 1.3	15.2 ± 1.3	25.1 ± 1.2	12.5 ± 1.4	12.4 ± 0.8	19.8 ± 1.6
60 d	5.8 ± 1.1	5.4 ± 1.2	6.7 ± 1.1	7.2 ± 1.4	4.8 ± 1.0	7.1 ± 1.1
cyfluthrin: 0 d	14.9 ± 1.3	15.2 ± 1.3	25.1 ± 1.2	12.5 ± 1.4	12.4 ± 0.8	19.8 ± 1.6
60 d	5.8 ± 1.1	5.4 ± 1.2	6.7 ± 1.1	7.2 ± 1.4	4.8 ± 1.0	7.1 ± 1.1

not change with time when incubated in water for up to 5 days, or after extraction with solvents such as ethyl acetate (**Table 3**). These analyses together indicate that the conditions used during analysis and extraction would not contribute to noticeable isomerization between diastereomers or enantiomers, and that the previous peak assignment was not biased as a result of isomerization.

Analysis of Water and Sediment Samples. The above methods were used for analyzing spiked water samples to evaluate method reproducibility and sensitivity. Quantification was made by assuming the same response factor for different stereoisomers from the same compound. Analysis that gave a signal-to-noise ratio of ≥ 3 was considered to be above the method detection limit (MDL). From **Table 4**, the MDL was 0.30 μg L^{-1} for CP diastereomers and 0.15 μg L^{-1} for CF diastereomers. The slightly lower detection limits for CF diastereomers may be attributed to its F substitution. Due to the prolonged retention times, the method sensitivity for detection of enantiomers was lower than that for diastereomers. The MDL ranged from 7.5 to 15 μg L^{-1} for CP enantiomers and from 3.5 to 7.5 μg L^{-1} for CF enantiomers (**Table 4**). The accuracy of analysis was good, as evident from the small standard deviations of replicated analyses (**Table 4**). It must be noted that the detection limit may be further improved when larger sample volumes are used, which may be achieved, for instance, by using solid phase extraction. Similar analysis was carried out also for sediment samples containing CP or CF residues. From the total concentration of diastereomers measured on day 0, the recovery given by the extraction and analytical procedures was 95.9% for CP, and 98.4% for CF (**Table 5**). The concentrations of diastereomers or enantiomers after 60 days of incubation were substantially smaller, indicating rapid pesticide degradation under the used conditions (**Table 5**). The standard errors for all analyses were relatively small, which, along with the relatively low detection limit, suggest that it is feasible to use the developed methods for analyzing SP diastereomers or enantiomers in environmental samples.

Our study showed that on a commonly used achiral column, all diastereomers of CP and CF were adequately separated. On a commercially available enantioselective column, complete separation of enantiomers occurred for both cis diastereomers and between cis and trans diastereomers, but not for enantiomers from the same trans diastereomer. Sensitivity and reproducibility analysis showed that the methods developed here may be used for analyzing SP diastereomers and enantiomers in aqueous and solid environmental samples. It also appears that elution of CP or CF diastereomers and enantiomers obey specific orders on selected GC columns. The relative elution order of SP isomers may be used for tentative peak identification in the absence of isomer standards. It will be of interest to validate the observations with other SPs and to apply the developed methods to other environmental samples such as soil and tissue samples.

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