

Enantioselective degradation of fipronil in Chinese cabbage (*Brassica pekinensis*)

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Abstract

Chiral pesticide enantiomers often show different bioactivity and residual toxicity, but this property is usually ignored when evaluating the environmental risk and public safety. In this study, a convenient and precise chiral method was developed and validated for measuring fipronil enantiomers in Chinese cabbage (*Brassica pekinensis*) based on a high-performance liquid chromatography (HPLC) using (*R,R*) Whelk-O 1 column. Then the proposed method was successfully applied to the study of enantioselective degradation of fipronil in Chinese cabbage under field conditions. The results showed that the degradation of the two enantiomers in Chinese cabbage was proved to be enantioselective and followed pseudo first-order kinetics ($R^2 \geq 0.98$). The (*R*)-enantiomer degraded faster than the (*S*)-enantiomer, resulting in the relative enrichment of (*S*)-enantiomer in residue. The detected metabolites MB46513 (desthio), MB45950 (sulfide) and MB46136 (sulfone) by GC–MS suggested that degradation was mainly contributed by oxidization, reduction and photo-degradation. Due to the more insecticide activity and lower mammalian toxicity of *S*-form, the higher concentration of *S*-fipronil may result in higher activity in crop protection and lower risk to environment and human beings compared to the recemate. This result should be considered in future environmental risk and food safety evaluation.

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1. Introduction

Chiral pesticides, accounting for more than a quarter of all pesticides used, have recently attracted increasing concern in the field of crop protection and environmental science (Liu, Gan, Schlenk, & Jury, 2005). As is well known, the enantiomers of a chiral pesticide have identical physical and chemical properties but always have different biological and physiological properties in asymmetry systems. The processes of absorption, distribution and degradation in organism and environment are often enantioselective (Monkiedje, Spiteller, & Bester, 2003;

Wang et al., 2005b; Zadra, Marucchini, & Zazzerini, 2002). Therefore, enantioselectivity is an important factor that should not be ignored concerning the effect of chiral pesticides on ecosystem. But in most cases, the enantiomers are always treated as one compound in traditional analysis. The adverse effects associated with particular enantiomers are neglected to be determined (Li, Zhang, Zhou, Gao, & Wang, 2002). Thus, the traditional risk evaluations of chiral pesticides residue are not reliable if enantioselective behaviors happened. Consequently, it is of great significance to develop enantiomeric analysis methods of chiral pesticides and investigate the different environmental behavior of the individual enantiomer to supply more accurate data for evaluating the environmental risk and food safety.

Fipronil, (*R,S*)-5-amino-1-(2,6-dichloro- α,α,α -trifluorotolyl)-4-trifluoromethyl-sulfinylpyrazole-3-carbonitrile, is a kind of phenylpyrazole insecticide discovered in 1987 by

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the French company Rhône-Poulenc Agro (Bobe, Coste, & Cooper, 1997). It is highly effective at low field application rates and selectively toxic toward pest insects because of its tighter affinity toward the GABA-regulated chloride channels of insects than the mammalian GABA receptors (Hainzl, Cole, & Casida, 1998). It was proved that fipronil is an effective insecticide using for Chinese cabbage (a major kind of vegetable in China) protection. Previous studies showed that fipronil degraded to MB46513 (fipronil-desulfinyl), MB45950 (sulfide), MB46136 (sulfone), and RPA200766 (amide) resulting from photolysis, reduction, oxidation and hydrolysis in the environment (Aajoud, Ravanel, & Tissut, 2003; Aajoud, Raveton, Aouadi, Tissut, & Ravanel, 2006; Bobe, Cooper, Coste, & Muller, 1998; Ngim, Mabury, & Crosby, 2000; Zhou, Lu, Liu, & Jay, 2004; Zhu, Wu, Guo, & Faithrest, 2004). Due to the presence of an asymmetric sulfoxide in the chemical structure, fipronil is chiral and has two enantiomers (Teicher, Kofoed-Hansen, & Jacobsen, 2003). This makes it necessary to study the individual bioactivities, toxicities and degradation behavior in the environment of the two enantiomers. Recent studies showed that the (*S*)-enantiomer obtained longer term control of ticks and lower level of emesis in the treated dogs than (*R*)-enantiomer (Huber, 2002). It was also reported that the (+)-enantiomer (*S*-form) of fipronil is about 3-times more toxic to *Ceriodaphnia dubia* than the (–)-enantiomer (*R*-form) (Konwick, Fisk, Garrison, Avants, & Black, 2005). However, within our existing knowledge, except for the study of enantioselective behavior of fipronil in rainbow trout (Konwick, Garrison, Black, Avants, & Fisk, 2006), few study on the enantioselective environmental behavior of fipronil enantiomers were done even though the environmental fate and the metabolic pathways of fipronil racemate (not differentiating the two enantiomers) were extensively studied.

HPLC with chiral stationary phases is an advisable choice for enantioseparation. In previous studies, fipronil enantiomers were always separated by polysaccharides chiral stationary phases (Teicher et al., 2003; Wang et al., 2005a; Wang, Liu, Lei, Jiang, & Zhou, 2006). But in this study, (*R,R*) Whelk-O 1 column was used to supply a new chiral method to separate this insecticide. (*R,R*) Whelk-O 1 column is one of the widely used Pirkle-type columns based on 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene. It had shown excellent enantiomeric recognition towards a large number of chiral compounds (Cancelliere, Acquarica, Gasparrini, Misiti, & Villani, 1999). Within our existing knowledge, this was the first report about the chiral separation of fipronil enantiomers on this Pirkle-type column.

Science the importance of chiral separation and necessary of the enantioselective degradation study, the aim of this work was to develop and validate chiral HPLC method for the analysis of fipronil enantiomers and investigate the possible enantioselective behavior in Chinese cabbage.

2. Materials and methods

2.1. Chemicals and materials

Analytical standard of fipronil racemate (purity: 96.1%, enantiomeric ratio ([*R*]/[*S*]):0.99), MB46513 (97.8%), MB45950 (97.1%), MB46136 (99.7%) were obtained from Institute for the Control of Agrochemicals, Ministry of Agriculture, China. Stock solutions of racemic fipronil were prepared in isopropanol at concentrations of 1–500 mg L⁻¹ for HPLC analysis and stock solutions of MB46513, MB45950 and MB46136 were prepared in acetone at concentrations of 0.1–50 mg L⁻¹ for GC–MS analysis. The solutions were stored at 4 °C until analysis. All reagents were of analytical grade (Beijing Chemical Reagent Co., China), redistilled and filtered through 0.45 μm filter membrane before use.

The degradation experiments were carried out with Chinese cabbage (*Brassica pekinensis*) in the experiment field of China Agricultural University (Beijing, China).

2.2. Chinese cabbage samples preparation

The homogenized Chinese cabbage samples (10 g in triplicate) were taken and transferred to 100-mL centrifuge tubes and mixed thoroughly by Vortex-Genie for 2 min after adding 30-mL acetonitrile. After centrifugation at 4000 r/min for 5 min, the liquid phase layer was transferred to 100-mL measuring cylinders with stoppers. The residues were then extracted with acetonitrile (30 + 20 mL) for two times as the steps above. Combine the liquid phase and add 2.0-g sodium chloride. The liquid phase layer was separated after a while of mixture shaking. Transfer acetonitrile layer to round-bottom flasks through anhydrous sodium sulfate and evaporated to near 1 mL by vacuum rotary evaporator at 45 °C, then blow it to dryness by nitrogen and dilute to 1 mL with petroleum ether/ethyl acetate (7/3, v/v).

The concentrated extract was then purified through a glass chromatography column (20 cm × 8 mm i.d.) successively packing with 1.5-g anhydrous sodium sulfate, 2.5-g neutral aluminum oxide + 0.2-g activated carbon and then 1.5-g anhydrous sodium sulfate. The column was pre-rinsed by 20-mL ethyl acetate and 20-mL petroleum ether. Load the extract and elute with 10-mL petroleum ether and then 30-mL of petroleum ether/ethyl acetate (7/3, v/v), discard the first 10 mL and collect the following eluate in a round-bottom flask. Evaporate to 1 mL using a vacuum rotary evaporator at 40 °C and evaporate under nitrogen to dryness. The residue was dissolved in 1 mL isopropanol for chiral HPLC analysis. Transfer 0.5 mL and blow it to dryness under nitrogen, then dilute it to 0.5 mL with acetone for GC–MS analysis.

2.3. Method validation for fipronil enantiomers by chiral HPLC and for metabolites by GC–MS

A series of standard solutions of racemic fipronil (2, 5, 50, 100, and 500 mg L⁻¹) and MB46513, MB45950,

MB46136 (0.1, 0.5, 2, 10 and 50 mg L⁻¹) for linearities were prepared in isopropanol and acetone, respectively. The relative standard deviations (RSD) were calculated at the calibration range. The run-to-run precisions of three concentrations (1, 5 and 50 mg L⁻¹) for individual enantiomer and three metabolites were determined by injections in six replicates and the day-to-day precisions were also tested over 6 days by six successive injections each day. Recovery assay was performed in untreated Chinese cabbage over three concentration levels (0.1, 1 and 10 mg kg⁻¹) for fipronil enantiomers and the metabolites. All the recovery validation studies were performed in triplicate ($n = 3$). The limit of quantification (LOQ) was considered to be the concentration that produced a signal-to-noise (S/N) ratio of 10:1.

2.4. Degradation experiments

Four plots of working areas for Chinese cabbage was chosen at the experiment field in China Agricultural University (Beijing, China), each with an area of 10 m². Three of the plots were applied with three replicates in order to avoid random error, and the other one was used as contrast (without fipronil). These plots had never been treated with fipronil for more than five years. Average temperatures were 25.0 ± 10.0 °C at Beijing, showing little difference throughout the course of the study, and no rainfall occurred after application of fipronil. Fipronil-SC was used as foliar spray at a rate of 240 g a.i. ha⁻¹ at 40 days after sowing. Three samples from each plot were collected at 0, 16, 40, 64, 112, 160, 196, 240 h after treatment. All the samples were rinsed with tap water for 10 min in order to remove the external residues and dust, homogenized in a blender immediately after being sopped up with filter paper and then stored at -22 °C until cabbage sample preparation.

2.5. Chiral high-performance liquid chromatography (HPLC)

Chiral separations of fipronil enantiomers were performed on (*R,R*) Whelk-O 1 column (250 mm × 4.6 mm i.d., Regis Technologies, Inc., Morton Groove, IL) using a JASCO 2000 HPLC system (JASCO Corporation, Japan) equipped with PU-2089 plus pump, UV-2075 plus ultraviolet detector, CD-2095 plus circular dichroism detector and 20-μL sample loop. The output signal was acquired and processed by Chrompass workstation. Scan of the circular dichroism (CD) spectrum of fipronil was carried out by Borwin Chromatography software. Column temperature was controlled by AT-930 heater and cooler column attenuator (Tianjin Automatic Science Instrument CO., LTD, China). Void times were determined using 1,3,5-tri-tert-butyl benzene. Injection volume was 20 μL. UV and CD detectors were performed at 225 nm and 234 nm, respectively. Capacity factor (k), separation factor (α) and resolutions (R_s) were calculated from the formula $k = (t - t_0)/t_0$,

$\alpha = k_2/k_1$, $R_s = 2(t_2 - t_1)/(W_1 + W_2)$. Where t was the retention time and t_0 was the void time at given conditions, k was retention factors and W was base widths (subscripts 1 and 2 represent the first and second eluted enantiomers, respectively). The enantiomeric ratio (ER) was defined as $ER = [R]/[S]$, where $[R]$ and $[S]$ are the concentrations of the (*R*)- and (*S*)-enantiomers. Triplicate injections were made for the measurements and the values were the mean of three injections.

Chromatographic conditions for method validation (including linearity, precision, recovery estimation and limit of quantification) and determination of fipronil enantiomers in Chinese cabbage were: *n*-hexane/isopropanol 95/5(v/v), UV detection at 225 nm and CD detection at 234 nm, 1.0 mL min⁻¹, 10 °C.

2.6. GC-MS analysis

The extracts for degradation experiment were then analyzed by Agilent GC 6890 with mass spectrometric detector Agilent MSD 5973B in order to evaluate the metabolites of fipronil in Chinese cabbage. GC-MS conditions were: electron impact mode, 70 eV; injector temperature, 250 °C; transfer line temperature, 280 °C; column, HP-5MS, 0.25 mm × 30 m × 0.25 μm; initial oven temperature, 50 °C for 1 min raised at 10 °C min⁻¹ to 100 °C, held for 1 min, raised at 20 °C min⁻¹ to 240 °C, held for 10 min; solvent delay, 5 min; carrier gas, helium at the flow rate of 1.0 mL min⁻¹; Inject volume, 1 μL. Under the conditions as described above, the retention time of fipronil, MB46513, MB45950 and MB46136 was 15.66, 14.54, 15.51, and 16.81 min, respectively. For single ion monitoring (SIM), four groups of ions were chosen: fipronil (351; 367 *uma*), MB46513 (333; 388 *uma*), MB45950 (351; 420 *uma*) and MB46136 (383; 452 *uma*). The metabolites quantities were estimated based on the chromatograms obtained in this SIM mode.

3. Results and discussion

3.1. Optimization of enantioseparation conditions

Investigation of the mobile phase composition and column temperature should always be included in the optimization of enantioseparation conditions. Mobile phase always plays the most important role for enantiomeric separation. In this work, Chiral separation of the two enantiomers was performed using *n*-hexane-polar organic alcohols mobile phase. Effects of ethanol, *n*-propanol, *iso*-propanol, *n*-butanol, *iso*-butanol and *n*-amylalcohol modifiers and their volume content on the separation of fipronil were studied and listed in Table 1. The two enantiomers of fipronil could achieve baseline separations using all the modifiers. Ethanol gave the most inefficient separation compared with other alcohols and isopropanol gave the best resolution. The optimal resolution was obtained using 5% isopropanol with the R_s value of 2.60.

Table 1
Influence of polar alcoholic modifiers in *n*-hexane on the enantioseparation of fipronil on (*R,R*) Whelk-O 1 column

Percentage of alcohol in hexane	Ethanol				<i>n</i> -Propanol			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
5	3.26	3.76	1.15	1.88	3.60	4.44	1.23	2.29
10	1.57	1.74	1.11	1.10	1.75	2.06	1.18	1.40
15	1.02	1.11	1.09	0.70	1.06	1.21	1.15	0.94
20	0.74	0.79	1.07	0.52	0.74	0.84	1.13	0.71
30	0.44	0.44	1	0	0.49	0.54	1.10	–
	<i>iso</i> -Propanol				<i>n</i> -Butanol			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
5	5.29	6.91	1.31	2.60	3.62	4.56	1.26	2.22
10	2.62	3.24	1.24	2.12	1.75	2.11	1.21	1.40
15	1.72	2.08	1.20	1.57	1.14	1.34	1.18	1.06
20	1.26	1.48	1.18	1.21	0.82	0.95	1.15	0.79
30	0.76	0.88	1.15	0.80	0.51	0.57	1.12	0.55
	<i>iso</i> -Butanol				<i>n</i> -Amyl alcohol			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
5	6.20	8.20	1.32	2.00	4.41	5.55	1.26	1.57
10	2.95	3.72	1.26	1.52	2.18	2.66	1.22	1.37
15	1.52	1.83	1.20	1.06	1.46	1.74	1.19	1.00
20	1.19	1.41	1.18	0.86	1.07	1.24	1.16	0.85
30	0.89	1.04	1.16	0.68	0.75	0.83	1.12	0.56

Flow rate: 1.0 mL min⁻¹, wavelength: 225 nm, room temperature.

Temperature is another significant factor both for optimization of the chiral separations and investigation of the mechanisms of chiral recognition. Increase in column temperature often causes short retention and worse resolution. The influence of column temperature on the chiral resolutions was investigated from 0 °C to 50 °C using hexane/*iso*-propanol (95/5) mobile phase in this study. The retention and separation decreased with increasing temperature as expected. But considering the influences of peak broaden at very low temperature, the optimal temperature in this experiment was 10 °C.

3.2. Determination of the elution order

For a chiral compound, the individual enantiomer corresponded to a specific CD signal at given wavelength and thus CD was frequently used to distinguish a pair of enantiomers. In this work, two eluted enantiomers of fipronil were determined by HPLC–CD method on (*R,R*) Whelk-O 1 column and distinguished by CD signals ((+) or (–)). The CD spectra of both enantiomers were also studied by stopped-flow scanning in the wavelength range of 220–420 nm. The result showed that the CD absorbance intensity was closely related to wavelength and the wavelength range of 220–280 nm was effective for identifying the enantiomers, and the wavelength of 234 nm was selected in the HPLC–CD method due to the maximum absorption. It was found that the CD spectra of the two enantiomers were approximately opposite to each other along the axis with the first eluted one was (+) and the second was (–) in the effective wavelength range.

The absolute configurations of the eluted enantiomers in this system were also confirmed referred to a previous research work. Teicher reported the chiral separation of fipronil on the Chiralcel OD column showing the first eluted enantiomer was (*R*)-form and the second was (*S*)-form (Teicher et al., 2003). Thus we conducted an experiment for chiral separation of fipronil on the Chiralcel OD column (250 mm × 4.6 mm i.d., Daicel Chemical Industries, Japan) by HPLC–CD using the same chromatographic condition as Teicher's. The elution order on Chiralcel OD column reversed to the order on (*R,R*) Whelk-O 1 column as Fig. 1 showed. Therefore, we can concluded the first eluted enantiomer was (*S*)-fipronil ((+) CD signal) and the second was (*R*)-fipronil ((–) CD signal) on (*R,R*) Whelk-O 1 column under *n*-hexane/*iso*propanol conditions.

3.3. Validation of the method

Validation of the developed method for determination of the fipronil enantiomers and metabolites in Chinese cabbage including linearity, precision, recovery and limit of quantification (the results were summarized in Table 2) showed the method was efficient and reliable.

3.4. Enantioselective degradation of fipronil in chinese cabbage

Enantioselective degradation of fipronil enantiomers was observed in this study and the typical HPLC chromatograms were shown in Fig. 2. During the period of treatment time, the concentration increased at the first

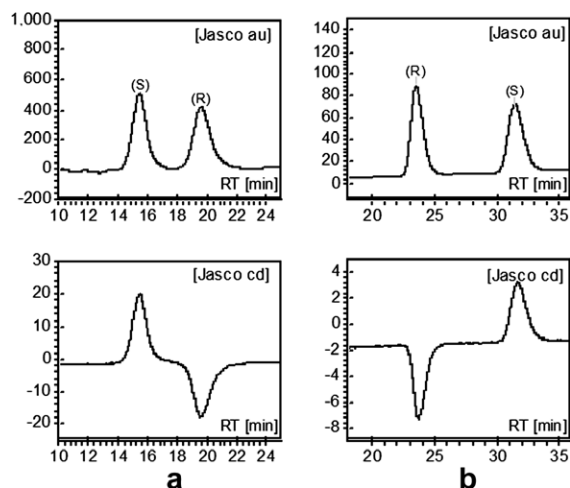


Fig. 1. UV and CD chromatograms for the chiral separations of fipronil, UV monitoring at 225 nm and CD at 234 nm, 1.0 mL min⁻¹; r.t. (a) (*R,R*) Whelk-O 1 column; *n*-hexane/isopropanol 95/5(v/v); (b) Chiralcel OD-H column; *n*-hexane/isopropanol 90/10(v/v).

Table 2
Method validation of fipronil enantiomers and its metabolites by HPLC and GC–MS

Compound	Concentration range (mg L ⁻¹)	Linear equation	<i>R</i> ²	Run-to-run precision (RSD, %)	Day-to-day precision (RSD, %)	LOQ (mg kg ⁻¹)	Recoveries ^a (%)			Method
							0.1 (mg kg ⁻¹)	1 (mg kg ⁻¹)	10 (mg kg ⁻¹)	
(<i>S</i>)-fipronil	1.0–250	$y = 0.6562x + 0.0334$	0.9997	0.2–0.9	2.5–4.9	0.05	87.71 ± 4.12	78.80 ± 3.08	90.89 ± 2.42	HPLC
(<i>R</i>)-fipronil	1.0–250	$y = 0.6633x - 0.4700$	0.9997	0.2–1.7	2.9–5.4	0.06	83.12 ± 4.38	83.64 ± 2.12	87.48 ± 3.34	HPLC
MB46513	0.1–50	$y = 88398x - 32374$	0.9995	0.4–1.9	1.7–4.6	0.008	90.29 ± 4.69	84.60 ± 3.58	87.42 ± 3.37	GC–MS
MB45950	0.1–50	$y = 91980x - 34301$	0.9996	0.4–2.2	2.3–5.8	0.010	88.44 ± 5.53	80.01 ± 3.51	84.23 ± 4.07	GC–MS
MB46136	0.1–50	$y = 88399x - 38716$	0.9996	0.5–0.8	2.6–6.1	0.010	85.68 ± 4.92	86.81 ± 2.85	79.06 ± 3.69	GC–MS

^a Recoveries represent the means recoveries ± SD (*n* = 3).

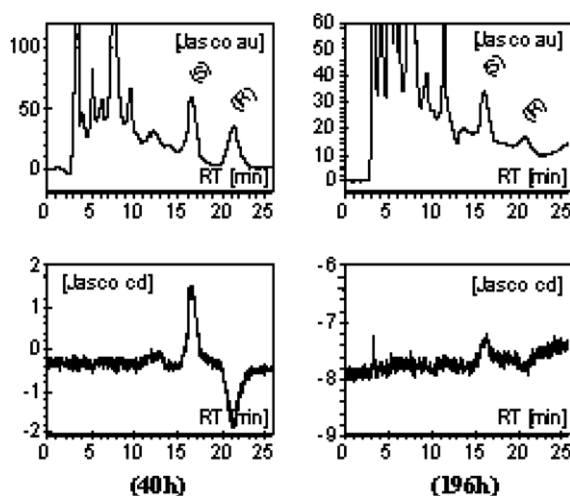


Fig. 2. Typical UV and CD chromatograms of extracts from Chinese cabbage fortified with racemic fipronil after 40 h and 196 h treatment hexane/isopropanol 95/5(v/v); 1.0 mL min⁻¹; 225 nm; 10 °C.

40 h due to the absorption process and then declined from 4.19 to 0.98 and 3.47 to 0.35 mg kg⁻¹ after 10 days treat-

ment for (*S*)- and (*R*)-enantiomers, respectively as the result of biodegradation (Table 3). The degradation pro-

Table 3
Concentrations of fipronil and its metabolites and enantiomeric ratio (ER = [R]/[S]) of (*R,S*)-fipronil in Chinese cabbage over treatment time^a

Time after treatment (h)	(<i>S</i>)-fipronil (mg kg ⁻¹)	(<i>R</i>)-fipronil (mg kg ⁻¹)	MB46513 (mg kg ⁻¹)	MB45950 (mg kg ⁻¹)	MB46136 (mg kg ⁻¹)	Enantiomeric Ratio (<i>R/S</i>)
0	–	–	–	–	–	0.989 ± 0.028
16	2.45 ± 0.23	2.17 ± 0.15	–	–	–	0.885 ± 0.057
40	4.19 ± 0.09	3.47 ± 0.21	–	0.22 ± 0.01	–	0.829 ± 0.039
64	3.20 ± 0.18	2.33 ± 0.13	0.33 ± 0.02	0.27 ± 0.01	0.43 ± 0.05	0.729 ± 0.063
112	2.30 ± 0.19	1.40 ± 0.08	0.39 ± 0.04	0.34 ± 0.04	0.54 ± 0.03	0.608 ± 0.041
160	1.84 ± 0.12	0.94 ± 0.06	0.43 ± 0.09	0.31 ± 0.06	0.83 ± 0.06	0.512 ± 0.075
196	1.39 ± 0.17	0.58 ± 0.04	0.36 ± 0.06	0.25 ± 0.03	0.53 ± 0.03	0.417 ± 0.036
240	0.98 ± 0.25	0.35 ± 0.08	0.24 ± 0.03	0.18 ± 0.02	0.37 ± 0.04	0.359 ± 0.067

^a Values represent the means ± SD ($n = 3$).

cess (from 40 h to 240 h) of fipronil enantiomers appeared to follow a pseudo first-order kinetic reaction, and the degradation rate constants and $t_{1/2}$ were determined using regression plots of $\ln(C_0/C)$ versus time (t) with the following equations:

$$\ln C_0/C = -k(t-t_0) \quad t_{1/2} = \ln 2/k = 0.693/k$$

where C_0 is the maximum concentration of fipronil (mg kg⁻¹) in Chinese cabbage ($C_0[S] = 4.19$ mg kg⁻¹, $C_0[R] = 3.47$ mg kg⁻¹); C is the concentration of fipronil (mg kg⁻¹) in Chinese cabbage at the time of t ; t is the treatment times in days; t_0 is the treatment time of maximum concentration ($t_0 = 1.67$ days); k is fipronil degradation rate constants (days⁻¹).

The data showed that the *S*-enantiomer degraded more slowly than the *R*-enantiomer with the equation $C = 5.5991e^{-0.1736t}$ ($R^2 = 0.9873$, $t_{1/2} = 3.99$ days) for (*S*)-fipronil and $C = 5.4963e^{-0.2754t}$ ($R^2 = 0.9927$, $t_{1/2} = 2.52$ days) for (*R*)-fipronil, resulting in relative enrichment of (*S*)-fipronil in Chinese cabbage. In order to compare the fate of the two enantiomers of fipronil, the ER ratios ([R]/[S]) in the plant extracts were investigated. The two enantiomers were present at the same concentration showing the initial ER value was approximately 1.0 but decreased steadily after treatment. The ER values ranged from 0.99

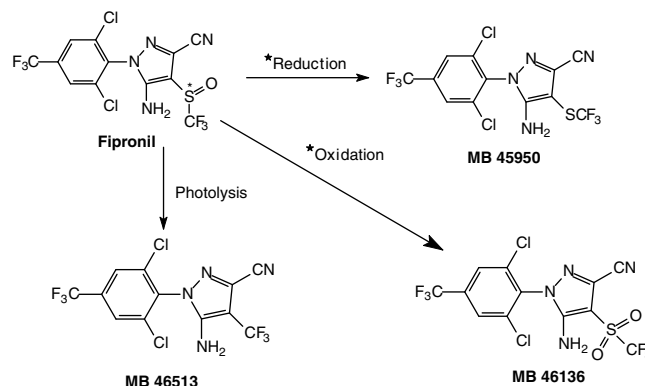
(ER value for Fipronil-SC) to 0.36 (composition *S* > *R*) in Chinese cabbage over time and were listed in Table 3 which indicated a preferential degradation of the *R*-enantiomer. Based on the equation proposed by Buser, Muller, Poiger, and Balmer (2002)

$$\ln(\text{ER}) = \ln(\text{ER}_0) + \Delta kt$$

where ER_0 was the initial enantiomer ratio $[R_0]/[S_0]$ (Because there was no fipronil in Chinese cabbage at 0 h, ER_0 in this experiment was the ER value for Fipronil-SC), and $\Delta k = (k_S - k_R)$ was the difference in the rate constants of the enantiomers.

A linear plot of $\ln(\text{ER})$ versus t was obtained with the equation $y = -0.103x - 0.01$ ($R^2 = 0.9951$).

The GC-MS was then used to determine the metabolites of fipronil in Chinese cabbage so as to elucidate the degradation pathway and the reason leading to enantioselective behavior. From the TIC chromatography and the MS fragmentation, the metabolites MB46513 (desthio-fipronil), MB45950 (sulfide-fipronil) and MB46136 (sulfone-fipronil) were observed during the treatment time, but RPA200766 was not found, indicating the majority of fipronil in Chinese cabbage was decomposed by reduction, oxidization and photodegradation process. The degradation pathways of fipronil in this study are presented in Fig. 3. This result



* Chiral centre of fipronil

* Biotricreaction

Fig. 3. Dynamics of fipronil enantiomers in Chinese cabbage.

about metabolites was similar as the previous report by Li, Deng, Luo, Liu, and Li (2005). Quantitative analyses of these three metabolites in the series of extracts were carried out by GC–MS with SIM chromatograms. Levels of metabolites residues detected in Chinese cabbage are showed in Table 3. The result strongly demonstrate that, under the tested conditions, biotic processes (reduction and oxidization) possibly caused by plant enzyme systems play an important role in the enantioselective degradation which may be responsible for the preferential biodegradation of one of the enantiomers, while photodegradation is another factor leading to dissipation of fipronil but it is an abiotic process which can not contribute to the enantioselective process.

4. Conclusion

This present study showed an effective chiral analysis method of insecticide fipronil and the proposed method was successfully applied to the study of enantioselective degradation of fipronil in Chinese cabbage under field conditions. The degradation of the two enantiomers of fipronil in Chinese cabbage was proved to be enantioselective. (*R*)-fipronil degraded faster than (*S*)-fipronil, resulting in the relative enrichment of (*S*)-form in residue. As mentioned above, the *S*-enantiomeric form is more active to insect and safe to mammalian than the *R*-form (Huber, 2002; Konwick et al., 2005). Therefore, the higher concentration level of *S*-form will lead to greater insecticide activity and lower toxicity, which would help protecting against pest in Chinese cabbage and ensuring food safety. However, due to the current lack of knowledge concerning the bioactivities and toxicities of individual enantiomer of fipronil and the potential ecotoxicological effects to benefit species, the possible risk about this chiral insecticide are still not clear. Further studies are needed to supply the detailed characters and determine the exact nature of the enantioselective processes of this chiral insecticide. This study showed an evidence of enantioselective behavior of a chiral pesticide and emphasized the importance to understand the detailed fate of the enantiomers so as to make more accurate environment and human health risk assessment.

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