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A novel LC-MS/MS method for enantioseparation tefluthrin via Box-Behnken Design and its stereoselective degradation in soil

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Abstract

A simple and eco-friendly dispersive solid-phase extraction (d-SPE) method coupled with ultra-high-performance liquid chromatography tandem triple quadrupole mass spectrometry (UPLC-MS/MS) was developed for the determination of the chiral pesticide tefluthrin in food and environmental samples. The response surface methodology (RSM) was applied to optimize separation conditions. The elution order of tefluthrin enantiomers was *Z-Cis*-(1*S*,3*S*)-(-)-tefluthrin, *Z-Cis*-(1*R*,3*R*)-(+)-tefluthrin on a Lux Cellulose-1 chiral column was identified via polarimeter and vibrating circular dichroism (VCD). The average recoveries in five matrices ranged from 76.9-107.6%, with intraday relative standard deviations (RSDs) less than 15.6% and interday RSDs less than 12.5% for two enantiomers. The enantioselective degradation was investigated via laboratory incubation experiments. Slightly enantioselective degradation was observed under aerobic conditions: (1*S*,3*S*)-tefluthrin degradation preferentially with the enantiomer fraction (EF) value 0.57 at 120 d of incubation. No remarkable enantioselective degradation was observed under anaerobic and sterile conditions. It was the first time that pyrethroid pesticides were determined on the enantiomers levels via UPLC-MS/MS. This novel method was successfully applied for the enantioselective analysis of tefluthrin enantiomers in authentic samples indicating its efficacy in investigating the environmental stereochemistry of tefluthrin in food web and environment. It is of crucial importance to improve risk assessment and regulation of chiral pesticides in agricultural system.

Keywords

40 Enantioseparation, Tefluthrin, Absolute configuration, Response surface methodology,

41 Enantioselective degradation

Introduction

It is commonly assumed that the significance of molecular chirality is widely recognized and discriminated in nature and artificial systems [1, 2]. Synthetic pyrethroids (SPs) are designed based on the structures of the pyrethrins, which are natural insecticides isolated from chrysanthemum flowers. SPs contain a special chiral structure and usually have two or more enantiomers; for example, bifenthrin should have eight enantiomers, but it only contains two isomers in the actual production process [3-5]. Research indicated that different enantiomers of chiral pesticides possessed the same physico-chemical properties but showed different characteristics in biology and toxicology behaviors [6-8]. Previous studies showed that lambda-cyhalothrin underwent enantioselective biodegradation in lizards and marine-derived fungi [9-12]. The behavior of SPs in *vivo* and in *vitro* might provide available evidence to better evaluate the environmental risk.

Tefluthrin (Figure 1), 2,3,5,6-tetrafluoro-4-methylbenzyl (1*RS*,3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2 dimethylcyclopropanecarboxylate, is a broad spectrum SPs insecticide that is a Na^+ ion channel modulator that holds the axon of a neuron open, resulting in paralysis and death. Tefluthrin has been wildly used to control underground pests, lepidopteran pests, and coleopteran and health pests on maize, cotton, and in the house. At same time, some countries and organizations have set maximum residual limits (MRLs), for example, almost all fruits and vegetables must be inferior to 0.05 mg/Kg in European Union. Furthermore, the MRL was set 0.5 mg/kg in kale and lettuce by Japan. Tefluthrin residue analytical methods focused on

64 animal fat, children's food and beeswax have been established [13-16]. Tefluthrin
65 contains two chiral carbon atoms, double bond and cyclopropane; theoretically, there
66 are eight stereoisomers, but its main ingredients in production are *Z*-, *cis*-isomers that
67 only have two enantiomers. Until now, racemic tefluthrin was widely produced and
68 sold on the market. There was no report about its toxicity, bioactivity or
69 environmental behavior at enantiomer level. Therefore, it is necessary to develop a
70 reliable, rapid, and sensitive chiral analytical method to thoroughly understand
71 enantioselective behavior, which can provide accurate evidence of risk assessment.

72 Optimization of instrument conditions by traditional methods, in which one
73 factor is changed while other factors are kept constant, is monotonous,
74 time-consuming, and expensive, especially when many variables must be considered
75 [18]. Response surface methodology (RSM) has been widely used to design
76 optimization programs, build mathematical models, determine effective factors, study
77 interactions, and search for best conditions on the field of chemical technology,
78 biological pharmacy and medical and health work [19-21]; it dismantles the barriers of
79 single-factor optimization. Box-Behnken design (BBD) was suitable for experimental
80 design of factors were less than 5 and levels were less than 3. Furthermore, number of
81 trials of BBD was less than Central Composite design (CCD) based on the same
82 factors. BBD was recommended owing to its economical advantage.

83 Soil play an important role in receiving and degrading organic pollutions. In this
84 study, the main objective was to develop an enantiomer analytical method of chiral
85 tefluthrin, and determine the enantioselective environmental behavior in soil. The

established method was efficient, sensitive and accurate to determine tefluthrin in food, vegetables and environmental matrices using UPLC-MS/MS with a Lux Cellulose-1 column. RSM was used to optimized enantioseparation experiments variables. The absolute configuration of tefluthrin enantiomers was confirmed by comparing experimental and calculated the vibrating circular dichroism (VCD) spectra. The extraction process was based on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method. This was the first time that an effective chiral separation method has been established for analysis of tefluthrin enantiomers in fruit, vegetables, and environmental samples, and the degradation was performed in soil under different conditions on the enantiomers levels. This study could provide scientific guidance for risk assessment and a basis for scientifically and rationally studying the difference in enantiomers.

Experimental

Reagents and Materials.

The tefluthrin (purity≥95.5%) was purchased from JinyuChem Co., Ltd. (Weifang, China). A pair of isomers (purity≥98%) was prepared by the Chiralway Biotech Co., Ltd. (Shanghai, China). HPLC grade methanol and acetonitrile were acquired from Merck (Darmstadt, Germany), and ultrapure water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). MS-grade ammonium formate was acquired from CNW Technologies Inc. (California, USA). Sorbents including primary secondary amine (PSA, 40-63 μm), C18 (40-63 μm), and graphitized carbon black (GCB, 38-128 μm) were obtained from ANPEL Laboratory Technologies Inc.

(Shanghai, China). All other chemical reagents were purchased from commercial sources. Standard solutions of racemic tefluthrin and enantiomers were prepared in HPLC-grade methanol and stored in the dark at 4 °C.

Chiral separation and MS conditions

The enantioseparation and analysis of tefluthrin were performed on a Waters ACQUITY UPLC^{TM} system (Milford, MA, USA) tandem triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) source using Lux Cellulose-1 chiral column (150 mm × 4.6 mm i.d., 3 μm, Phenomenex, USA). The mixture of solvent A (5 mM ammonium formate in methanol) and solvent B (5 mM ammonium formate in water) in 83:17 (v/v) used as mobile phase with flow rate 0.23 mL min^{-1} at 30 °C.

MS analyses were carried out in multiple reaction monitoring (MRM) mode employing ESI in positive mode with capillary voltage 3.6 kV; source temperature and desolvation temperature were 120 °C and 300 °C, respectively. The 99.95% nitrogen was used as cone gas flow at 50 L h^{-1} and desolvation gas flow at 800 L h^{-1}. The 99.99% argon was used as collision gas was with a pressure of 2×10^{-3} mbar in the T-Wave cell. The characteristic product ions arising from ammonium adduct $[M+NH_4]^+$ (*m/z* 436 > 177) was extracted for quantitative and qualitative determination of tefluthrin enantiomers with the cone voltage and collision energy set as 20 v and 34 v, respectively. The Masslynx NT v.4.2 (Waters, USA) software was used to collect and analyze the data.

Separation condition optimization

To study the effect of single factors on the chiral separation, flow rate, temperature, and mobile composition were tested individually while keeping other instrument conditions at a constant level. The separation parameters, including capacity factor (k), separation factor (α), and resolution (Rs), were calculated to evaluate the effect of enantioseparation under different conditions. The enthalpy ($\Delta\Delta H°$) and entropy ($\Delta\Delta S°$) variation between enantiomers were also calculated using the following Van't Hoff equations.

$$\ln k = -\Delta H°/\mathrm{RT} + \Delta S°/\mathrm{R} + \ln \Phi \quad (1)$$

$$\ln \alpha = -\Delta\Delta H°/\mathrm{RT} + \Delta\Delta S°/\mathrm{R} \quad (2)$$

An RSM design was carried out to explain interaction effects between single factors and multifactors on enantioseparation and define an optimal formulation. It is well known that BBD gets the upper hand over the traditional "one-factor-at-a-time optimization experiments". In this study, BBD was used to optimize the separation conditions for Rs and retention time of tefluthrin enantiomers using the Design-expert 8.0.6 trial software. Based on the single factor experimental results, a BBD with three factors and three levels (including five replicates at center point) was carried out. A total of 17 randomized experiences were run in quintuplicate in the central point (See the Supporting Information Table S1). The process can be described by the quadratic model using equation:

$$Y=b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (3)$$

where Y stands for the predicted response, b_n are quadratic coefficients, and X_1, X_2, X_3 are the studied variables.

Determination of specific optical rotation.

The specific optical rotation of tefluthrin enantiomers was measured by Autopol Ⅳ polarimeter (Rudolph Research Analytical., NJ USA) at 589 nm. Each enantiomer dissolved in methanol with a concentration of 0.01g mL^{-1} was determined at 20 °C.

Confirmation of absolute configuration

Experimental infrared spectroscopy and VCD spectroscopy for tefluthrin enantiomers were performed using Bruker FTIR Vertex50 spectrometer equipped with a Bruker PMA50 VCD module (Karlsruhe, German) at room temperature. The spectra were collected with a total acquisition time of 5 h (5 times × 1 h) using a path length of 0.1 mm potassium bromide plates with a resolution of 4 cm^{-1}.

Geometry optimizations and IR and VCD spectra calculations for tefluthrin enantiomers were performed via density functional theory using the B3LYP function with the 6-311+G (2d,p) basis set [17,18]. Frequency calculations were carried out at the same level to confirm that the stationary points are the minima with zero imaginary frequency. The different conformations of tefluthrin were searched on the conformation bearing the lowest free energy, and the VCD calculation was performed. To create the final spectra, each intensity of the line spectra obtained by the DFT calculations were convoluted with a Lorentzian band shape with half-width at half-height of 4 cm^{-1}. All calculations were performed with the Gaussian 09 software package [19]. The absolute configuration of a pair of enantiomers was confirmed by comparing the similarity of the experimental VCD spectra and calculated VCD spectra.

Chiral Stability

To exclude the epimerization occurrence of chiral SPs insecticides in acetonitrile, methanol, and water, the enantiomer standard solution of 1 mg L^{-1} in water, methanol, and acetonitrile was stored in 4 °C and 25 °C for 180 d. The samples were collected at 0, 1, 3, 7, 14, 30, 60, 120, and 180 days, and filtered using a 0.22-μm nylon syringe filter before injecting the UPLC-MS/MS analysis.

Sample Preparation.

The soil sample was collected from a depth of 0-15 cm from a paddy field in Nanjing, China. The physicochemical property of test soil was summarized in the Table S2. The soil was thoroughly blended, air-dried, and sieved. The apple, pear, lettuce, and tomato were purchased from the organic food window of the local supermarket, and minced by a homogenizer. The samples were verified to be free of tefluthrin.

Extraction and Clean up for soil, apple, and pear.

The extraction process was based on the QuEchERs method. A.mple (soil, apple, and pear) was weighed into 50 mL polytetrafluoroethylene (PTEF) centrifuge tube, and then 5 mL water and 10 mL acetonitrile were added. The tube was immediately capped and shaken by high-speed vortex mixer at 2500 rpm for 5 min and ultrasonication for 10 min. The 3 g sodium chloride and 1 g anhydrous magnesium sulfate were added into the mixture, the tube was again shaken for 3 min, and the samples were centrifuged at 4000 rpm for 5 min, 5 mL of the upper layer was added into a 15 mL centrifuge tube containing 100 mg GCB and 1 g anhydrous magnesium sulfate. The tube was shaken vigorously for 1 min and centrifuged at 4000 rpm for 5

196 min. Next, 2 mL of the upper layer was transferred into a 4 mL centrifuge tube and
197 dried with nitrogen. Finally, the residual was dissolved in 1 mL mobile phase and
198 filtered using a 0.22-μm nylon syringe filter before injecting for the UPLC-MS/MS
199 analysis.
200 *Extraction and Clean up for tomato and lettuce.*
201 The process was similar to extraction and cleanup for the above samples, a 10 g
202 matrix (tomato and lettuce) was weighed into a 250 mL flask, and a mixture of 10 mL
203 water and 20 mL acetonitrile was added. The flask was shaken for 1 h in an oscillation
204 table at 250 rpm before the liquid mixture was transferred into a 100 mL graduated
205 cylinder with stopper containing 2 g sodium chloride. The supernatant acetonitrile (6
206 mL) was transferred into a 15 mL centrifuge tube containing 100 mg GCB and 1 g
207 anhydrous magnesium sulfate. The tube was vigorously shaken for 1 min and
208 centrifuged for 5 min at 4000 rpm. The 4 mL of liquid supernatant was evaporated to
209 dryness at 40 °C, and then the residual was dissolved in 1 mL of the mobile phase and
210 filtered using a 0.22 μm nylon syringe filter before injecting the UPLC-MS/MS
211 analysis. The typical chromatograms of blank and spiked samples were shown in
212 Figure S1.
213 **Method Validation.**
214 The specificity, LOD, LOQ, accuracy, and precision were used to evaluate the
215 performance of the method. Control samples (soil, apple tomato, pear, and lettuce)
216 were analyzed to confirm the absence of interfering substances at the retention time of
217 target chemicals. The linearity was verified for both solvent and matrix-matched

218 calibration curves at seven concentrations ranging from 10 to 1000 μg L^{-1}. The
219 linearity of the solvent and the different matrix-matched calibration curves were
220 determined based on the peak areas and the concentrations of target analytes. The
221 equation of matrix effect was defined as

222 $$\text{Matrix effect (\%)} = \frac{\text{the slope of matrix matched curve - the slope of solvent curve}}{\text{the slope of solvent curve}} \times 100\%. \quad (4)$$

223 The matrix-dependent LODs and LOQs of tefluthrin enantiomers in the fruits,
224 vegetables, and environmental samples were determined at concentrations that
225 produced signal-to-noise (S/N) ratios of 3 and 10, respectively.

226 The recovery experiments were employed to deduced the accuracy and precision
227 of the method. The control samples in quintuplicate were spiked with three different
228 concentrations (0.05, 0.5, 2.5 mg kg^{-1}) of five matrices, vortexed for 30 s and
229 incubated overnight. All spiked samples were prepared for three consecutive days and
230 tefluthrin enantiomers were extracted and purified according to the above procedure.
231 The recoveries, intraday RSDs, and interday RSDs were used to evaluate the accuracy
232 and precision.

233 **Stereoselective degradation in soil**

234 Incubation experiments were performed separately under aerobic, anaerobic and
235 sterile conditions. The experiments of racemate was to study the degradation tendency
236 and the enantiopure isomers treatments were used to evaluated the enantiomerization.

237 The sterile soil sample was prepared by sterilized two periods of 60 min, 121 °C
238 moist heat sterilization with a 24 h interval between autoclave treatments. [19] The
239 spiked concentration of racemate was 2 mg kg^{-1} and 1 mg kg^{-1} for enantiopure

tefluthrin.

Aerobic and sterile incubation experiments were performed in 50 mL PTEF centrifuge tubes covered with air-permeable, sterile cotton plugs. Accurately measured 5 g of the soils into PTEF centrifuge tubes. The water content was adjusted 60% using deionized water (contain 0.2% sodium azide solution for sterile conditions) of the saturation holding capacity and incubated at 25 °C in the dark was referenced Pan [20] and Zhang [21]. Triplicate samples were conducted at 0, 1, 3, 7, 14. 21, 35, 60, 90, 120 d for tefluthrin, and immediately transferred into a freezer (-20 °C) and stored for later analysis.

In anaerobic incubation experiments, deionized water, previously purged with N_2 to remove O_2, was added to each tube to form a 1 cm water layer on surface, and then the tubes were filled with N_2 and sealed immediately. Finally, the soil samples was treated the same as those described in under aerobic conditions.

The first-order kinetic Eq. (5) was used to declare the degradation kinetic of tefluthrin and enantiomers. The half-life ($T_{1/2}$) was calculated with the following Eq. (6), and EF value was defined in Eq. (7).

$$C_t = C_0 e^{-kt} \quad (5)$$

$$T_{1/2} = ln2/k = 0.693/k \quad (6)$$

$$EF = [R] / ([R]+[S]) \quad (7)$$

where C_0 and C_t are the initial concentration and the concentration at time in soil, respectively. k is the degradation rate constant. [R] and [S] are the contration of the (1R,3R)- and the (1S,3S)-enantiomers, respectively.

Results and discussion

MS Analysis

Experiments were performed to optimize the MS/MS conditions for tefluthrin, which forms abundant adduct ions in the mass spectra, such as $[M+Na]^+$, $[M+K]^+$, or $[M+NH_4]^+$, when undergoing ionization in the positive mode. The use of mobile phase containing ammonium formate helped to favor the formation of $[M+NH_4]^+$ for tefluthrin. Higher abundant product ions of *m/z* 177 for fragmentation of $[M+NH_4]^+$ (*m/z* 436) were excavated by manual tuning mode. The $[M+NH_4]^+$ (*m/z* 436 > 177) was selected as quantitative and qualitative determination ions of tefluthrin enantiomers, in agreement with the literature [14-16]. Different buffer concentrations (1, 2, 5 and 10 mM) were investigated, and the MS signal response was the highest at 5 mM.

The specific optical rotation and absolute configuration of tefluthrin enantiomers

The specific optical rotations were determined as Peak 1 $[\alpha]_D^{20}$ = -16.6° (methanol, c=1.13) and Peak 2 $[\alpha]_D^{20}$ = + 16.8° (methanol, c=1.15). As shown in Figure 2, the dashed lines indicated the calculated VCD spectra of the conformation bearing the lowest free energy, and the experimental VCD spectra of enantiomers were drawn using continuous lines. The experimental VCD spectra of tefluthrin enantiomers better agreed with the calculated VCD spectrum of (1*S*,3*S*) and (1*R*,3*R*). Therefore, the elution order of tefluthrin on the Lux Cellulose-1 was determined as *Z*-(1*S*,3*S*)-*cis*-(-)-tefluthrin and *Z*-(1*R*,3*R*)-*cis*-(+)-tefluthrin. VCD would affect the tendency of definiting absolute configuration for its advantage of giving more

information.

Sample Extraction and Purification

The QuEChERS method is a major development for sample preparation in pesticide residue analysis. Inorganic buffer salts play an irreplaceable role in this whole process; for example, anhydrous magnesium sulfate is soluble in water and prompts partitioning of the pesticides into the organic phase. The application of adsorbents in the cleanup procedure makes the QuEChERS method an attractive alternative approach [22]. PSA, C18, and GCB were used to remove the interfering substances, and the experiments indicate that after purification with C18 and PSA, and dispersive solid-phase extraction (d-SPE), poor recovery (<60%) was obtained for lettuce samples. Based on the higher recovery, GCB was selected to purify the lettuce matrix in place of C18 and PSA. The typical chromatograms were shown in Figure S1.

Method validation

Specificity, linearity, LOD, LOQ, and Matrix Effect

The specificity of the proposed method was detected by preparing blank samples according to the above procedures, and no interference was shown at the retention time. Good linearities ($R^2 \geq 0.9903$) were obtained for enantiomers; LODs and LOQs for tefluthrin enantiomers are summarized in Table 1. The LODs for tefluthrin enantiomers were estimated at 0.0029~0.0067 mg kg^{-1}, and the corresponding LOQs were 0.0101~0.0227 mg kg^{-1}.

One of the major drawbacks in pesticide analysis is that the response can be suppressed or enhanced by the presence of matrix components. Table 1 also shows the

306 matrix effect for each enantiomer of tefluthrin. The results show that significant
307 matrix enhancement effects existed between (1*R*,3*R*)-tefluthrin and (1*S*,3*S*)-tefluthrin
308 enantiomers. As a result, a calibration was performed for each enantiomer using the
309 external matrix-matched standards to eliminate the matrix effect and to obtain more
310 realistic results in the 5 matrices.

311 *Precision and Accuracy*

312 The recoveries and RSDs of tefluthrin enantiomers in quintuplicate at three
313 concentrations and on three consecutive days are shown in Table 2. From Table 2, the
314 proposed method possesses satisfactory mean recoveries values (76.9%–107.6%) and
315 precision with all RSDs values below 15.6%. For (1*S*,3*S*)-tefluthrin and
316 (1*R*,3*R*)-tefluthrin, the mean recoveries ranged from 78.8% to 107.6% and 76.9% to
317 105.4%, respectively, with the intraday RSDs of 0.64% to 15.6% (n=5) and 1.7% to
318 10.7% (n=5). The results indicate that this method can reach expectation of precision
319 and accuracy for the enantiomeric analysis of tefluthrin in 5 matrices.

320 **Stereoselective degradation of tefluthrin in soil**

321 *Stereoselective degration in soils under aerobic conditions*

322 The dissipation of tefluthrin in paddy soil was studyed under aerobic conditions
323 (Figure 3a). The degradadation of different isomers followed the first-order kinetic
324 model (R^2 = 0.9716-0.9737), and the half-lives, *k* and R^2 are listed in Table 3. The
325 half-lives of (1*S*,3*S*)-tefluthrin and (1*R*,3*R*)-tefluthrin were 63.4 ± 5.3 and 77.6 ±
326 4.5 d, respectively. During the incubation, the degradation rate of (1*R*,3*R*)-tefluthrin
327 tend to be slower than (1*S*,3*S*)-tefluthrin. The calculated EF value was used to

328 evaluate the stereoselective dissipation, EF slightly increased after 60 d (Figure 3d).
329 The results indicate that (1*S*,3*S*)-tefluthrin were preferentially degraded under aerobic
330 conditions. The concentration of (1R,3R)-tefluthrin was higher than (1S,3S)-tefluthrin
331 in soil under aerobic condition would lay the foundation for understanding the toxicity
332 for terrestrial biota.
333 *Stereoselective dissipation in soils under anaerobic conditions*
334 The concentration of isomers decreased about 15%-18% and the degradation all
335 followed the first-order kinetics (0.962-0.9794), *k* and half-lives were summarized in
336 Table 3. Notably, the degradation rate of tefluthrin isomers under anaerobic
337 conditions (Figure 3b) was slower than aerobic conditions. The half-lives of two
338 stereoisomers were 351.7 ± 13.5 and 357.2 ± 9.6 d, was 6 times than under aerobic
339 conditions. At the same time, the EF value was shown in Figure 3d to evaluate the
340 stereoselective dissipation, EF values changed around 0.5 during the whole incubation,
341 indicating that no significant enantioselectivity was observed under anaerobic
342 conditions and anaerobic bacteria made a contribution to degradation.
343 *Stereoselective dissipation in soils under sterile conditions*
344 During the incubation process, the concentration of tefluthrin slightly changed in
345 autoclaved soil compared with that under aerobic conditions. In sterile conditions,
346 10%-12% of tefluthrin isomers was degraded. This indicated that tefluthrin could be
347 degraded without microorganisms (Figure 3c). In addition, no stereoselective
348 dissipation was observed under these conditions (EF≈0.5, Figure 3d).
349 The microbial community plays an important contribution to degrade the

350 contaminant in soil samples [23,24]. In this study, the result indicated that the main
351 reason causing the dissipation of tefluthrin stereoisomers was the microbial activity
352 rather than abiotic factors. Interestingly, the degradation rate of tefluthrin isomers
353 under aerobic condictions was significantly faster than under other conditions. In
354 addition, the concentration of (1*R*,3*R*)-tefluthrin was slightly higher than that of the
355 (1*S*,3*S*)-tefluthrin under aerobic condition. It may be that the oxygen status affected
356 the makeup of microorganism population, hereby influenced the degradation rate and
357 stereoselectivity [20]. Compared to aerobic condictions, autoclaved soil samples
358 exhibited emaciated degradation ability to tefluthrin. Although the underlying
359 processes are unknown, this result may be explained by the activation/inhibition of
360 enzymes [25-27]. Therefore, increasing the microbial abundance may reduce the
361 environmental risks of tefluthrin. At the same time, this also lay the groundwork for
362 terrestrial environmental risk evaluation of tefluthrin stereoisomers.

363 *Stability of tefluthrin stereoisomers*

364 Incubating the enantiopure isomers under aerobic conditions was to study the
365 interconversion. During the 120 d of incubation, no enantioselective transformation
366 was observed. As shown in Figure S2, there was no significant change between the
367 initial concentration and measured concentration at different times of a pair of
368 enantiomers in acetonitrile, methanol, and water during the 6 months. The data
369 indicated that the chiral configuration was stable in soil and solvents.

370 **Abbreviations Used**

371 UPLC-MS/MS, ultraperformance liquid chromatography tandem mass spectrometry;

RSM, response surface methodology; VCD, vibrating circular dichroism; RSD, relative standard deviations; QuEChERS, quick, easy, cheap, effective, rugged and safe; *k*, capacity factor; *α*, separation factor; *Rs*, resolution; EF, enantiomer fraction.

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Supporting Information description

Effects of the flow rate, mobile phase compositions, and temperature on the enantioseparation parameters; separation condition optimization via RSM with BBD; stability of enantiomers; and anova of the regression model for the response variables.

Conflict of interest

The authors declare that they have no conflict of interest.

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476

477 **Figure captions**

478 **Figure 1** The chemical structure of tefluthrin enantiomers and typical chromatograms.

479 **Figure 2** Calculated VCD spectrum and experimental VCD spectrum.

480 **Figure 3** Degradation of tefluthrin enantiomers in Nanjing soil. a. Rac-tefluthrin
481 under aerobic condition; b. Rac-tefluthrin under anaerobic condition; c. Rac-tefluthrin
482 under sterile condition; d. EF value of tefluthrin stereoisomers under three conditions**.**

483

484 **Table 1** Linear regression parameters, LOD, and LOQ for the tefluthrin enantiomers
485 in different matrices

Compound	Matrix	Linear range (μg L^{-1})	Regression equation	R^2	Matrix effect (%)	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
	solvent	10 ~ 1000	y=1853.4x-188.8	0.9992		0.0029	0.0101
	tomato	10 ~ 1000	y=4290.2x+330.3	0.9986	131.4	0.0032	0.0114
(1*R*,3*R*)-	lettuce	10 ~ 1000	y=4001.3x-539.2	0.9901	115.8	0.0045	0.0146
tefluthrin	pear	10 ~ 1000	y=4692.9x+144.9	0.9936	153.2	0.0037	0.0125
	apple	10 ~ 1000	y=3028.8x+426.4	0.9904	63.4	0.0038	0.0137
	soil	10 ~ 1000	y=6341.3x-316.7	0.9935	242.1	0.0041	0.0138
	solvent	10 ~ 1000	y=1846.9x-187.9	0.9997		0.0036	0.0126
	tomato	10 ~ 1000	y=4327.5x+352.3	0.9993	134.3	0.0039	0.0131
(1*S*,3*S*)-	lettuce	10 ~ 1000	y=3997.9x-507.4	0.9972	116.5	0.0032	0.0117
tefluthrin	pear	10 ~ 1000	y=4731.2x+160.7	0.9941	156.2	0.0043	0.0144
	apple	10 ~ 1000	y=3232.7x+412.3	0.9981	75.0	0.0067	0.0227
	soil	10 ~ 1000	y=6452.3x-280.9	0.9966	249.4	0.0046	0.0151

486

487 **Table 2** Accuracy and precision of the method in the 5 matrices

Compound	Matrix	Spiked level (mg kg^{-1})	Intraday (n=5) Day1 Mean recovery (%)	Day1 RSD (%)	Day2 Mean recovery (%)	Day2 RSD (%)	Day3 Mean recovery (%)	Day3 RSD (%)	Interday (n=15) RSD(%)
(1*S*,3*S*) - Tefluthrin	tomato	0.05	95.1	1.3	98.3	4.8	98.3	4.8	4.5
		0.5	78.8	4.6	84.8	7.6	82.3	9.4	9.2
		2.5	95.2	6.6	106.9	6.9	100.1	6.7	6.0
	lettuce	0.05	101.7	2.6	101.8	2.4	100.2	2.5	2.5
		0.5	83.9	8.9	86.5	6.9	88.9	11.1	7.4
		2.5	80.0	6.5	79.9	6.8	80.8	5.5	5.3
	pear	0.05	99.2	6.3	93.2	2.8	90.4	5.4	5.4
		0.5	87.6	10.4	102.3	2.8	93.7	5.7	5.7
		2.5	101.7	4.5	84.3	3.6	98.9	8.4	8.4
	apple	0.05	104.3	5.5	107.6	2.7	105.8	4.6	4.0
		0.5	100.0	2.9	88.2	15.6	93.4	15.3	14.0
		2.5	97.2	0.6	97.4	1.2	97.1	1.0	0.9
	soil	0.05	89.5	3.3	96.3	2.6	82.0	10.1	8.0
		0.5	87.4	1.1	90.2	1.9	86.8	2.1	2.0
		2.5	88.9	1.1	89.3	2.8	89.6	3.3	2.4
(1*R*,3*R*) - Tefluthrin	tomato	0.05	96.2	2.9	97.3	3.9	98.7	1.6	3.2
		0.5	76.9	4.3	86.1	9.4	84.0	10.7	9.9
		2.5	90.0	8.2	103.8	2.0	99.5	7.0	7.3
	lettuce	0.05	99.1	2.6	98.5	3.1	97.3	1.0	2.5
		0.5	85.5	14.2	89.7	12.2	94.4	5.5	11.6
		2.5	87.9	14.6	90.8	14.5	92.8	9.9	12.5
	pear	0.05	90.7	4.8	93.6	7.2	91.4	2.4	2.6
		0.5	93.2	5.7	105.4	5.8	96.4	2.8	7.6
		2.5	81.0	5.2	88.9	3.5	86.7	4.9	6.6
	apple	0.05	94.9	7.1	99.9	6.8	97.8	9.9	7.4
		0.5	98.6	4.9	93.7	8.7	96.6	8.3	10.8
		2.5	100.0	1.6	102.9	2.3	101.8	1.7	2.2
	soil	0.05	104.0	14.1	81.2	6.2	93.1	4.6	12.1
		0.5	102.0	9.3	105.4	3.7	105.4	3.6	2.5
		2.5	97.4	1.4	98.4	2.1	98.4	4.8	0.6

488

489 **Table 3** Parameters for the dissipation of tefluthrin stereoisomers in soil.

Incubation condition	Isomers	k (d^{-1})	Half-lives (d)[a]	R^2	EF (End point)
aerobic	(1*S*,3*S*)	0.011	63.4 ± 5.3	0.9737	0.57
	(1*R*,3*R*)	0.009	77.6 ± 4.5	0.9716	
anaerobic	(1*S*,3*S*)	0.00197	351.7 ± 13.5	0.962	0.49
	(1*R*,3*R*)	0.00194	357.2 ± 9.6	0.9794	

490 a Value represent mean ± SD.

491

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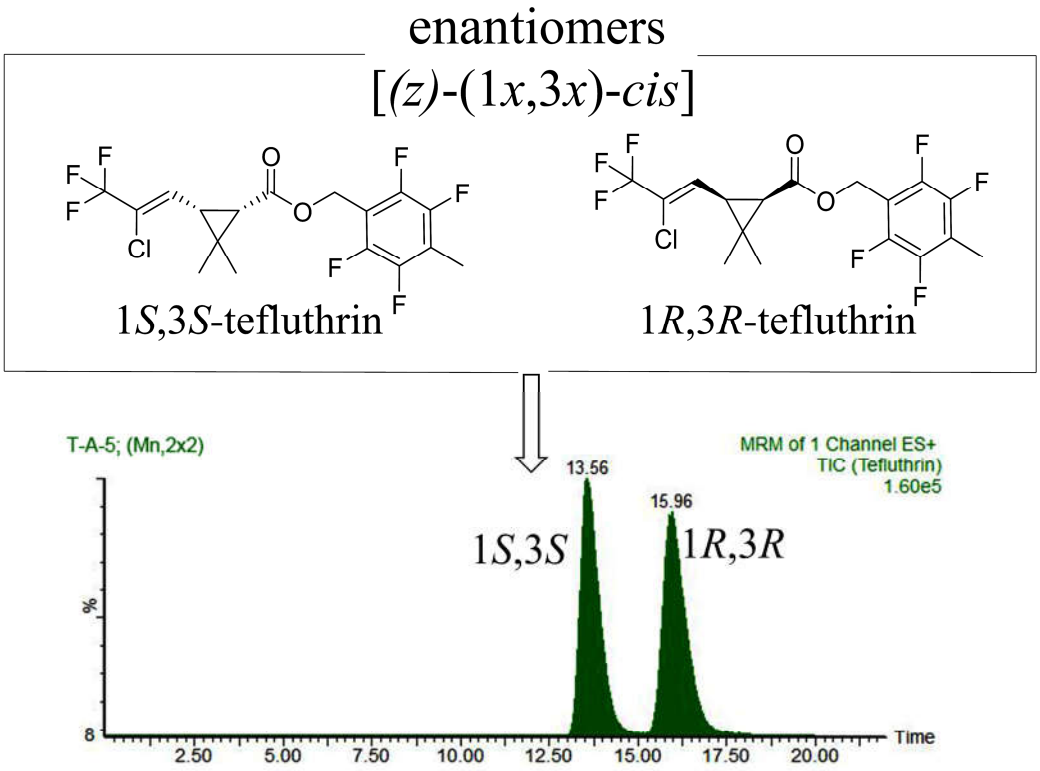

493

494 **Figure 1** The chemical structure of tefluthrin enantiomers and typical chromatograms.

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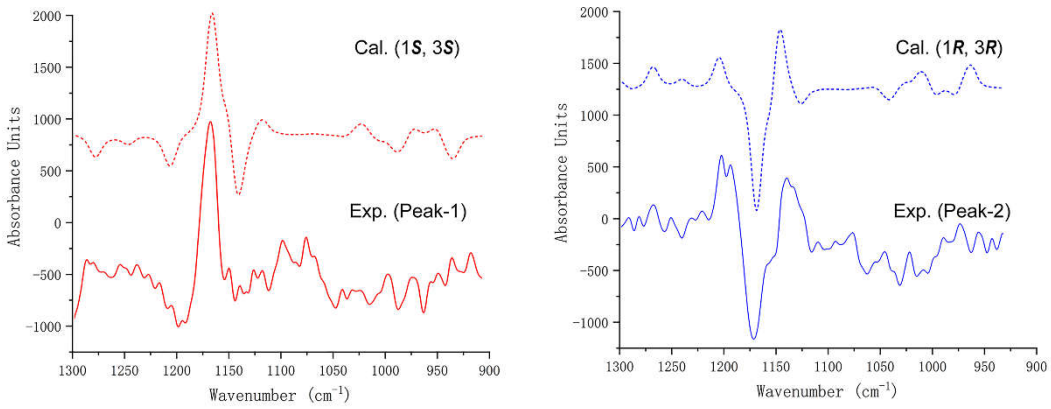

497

498 **Figure 2** Calculated VCD spectrum and experimental VCD spectrum.

499

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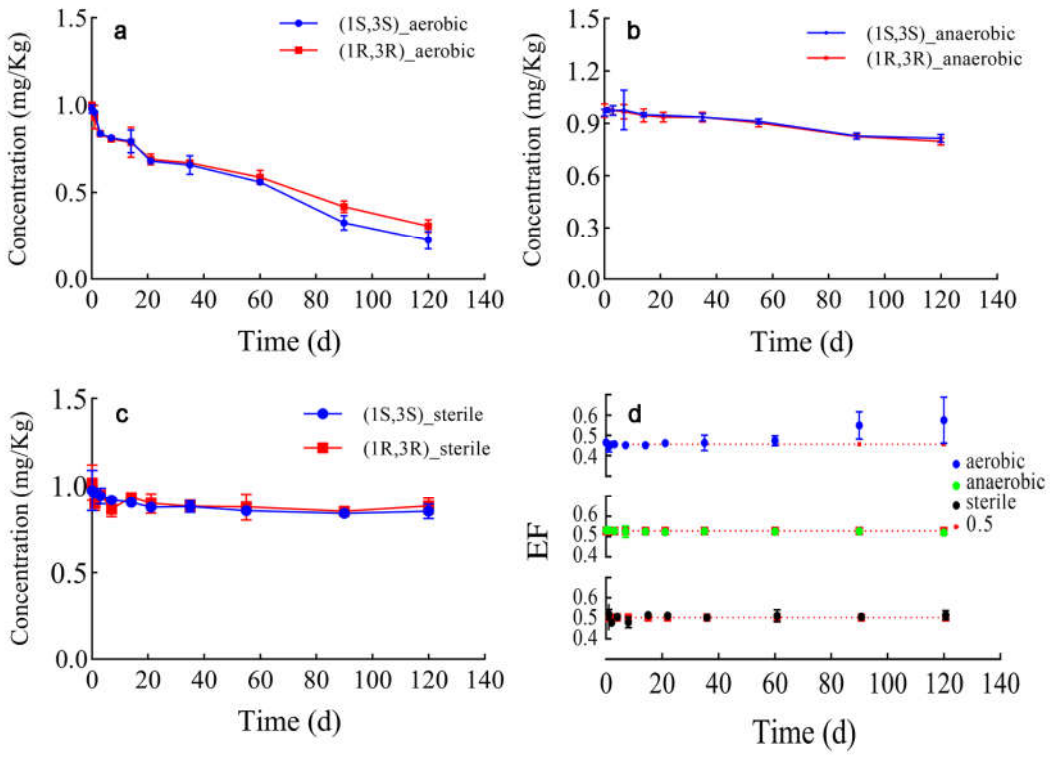


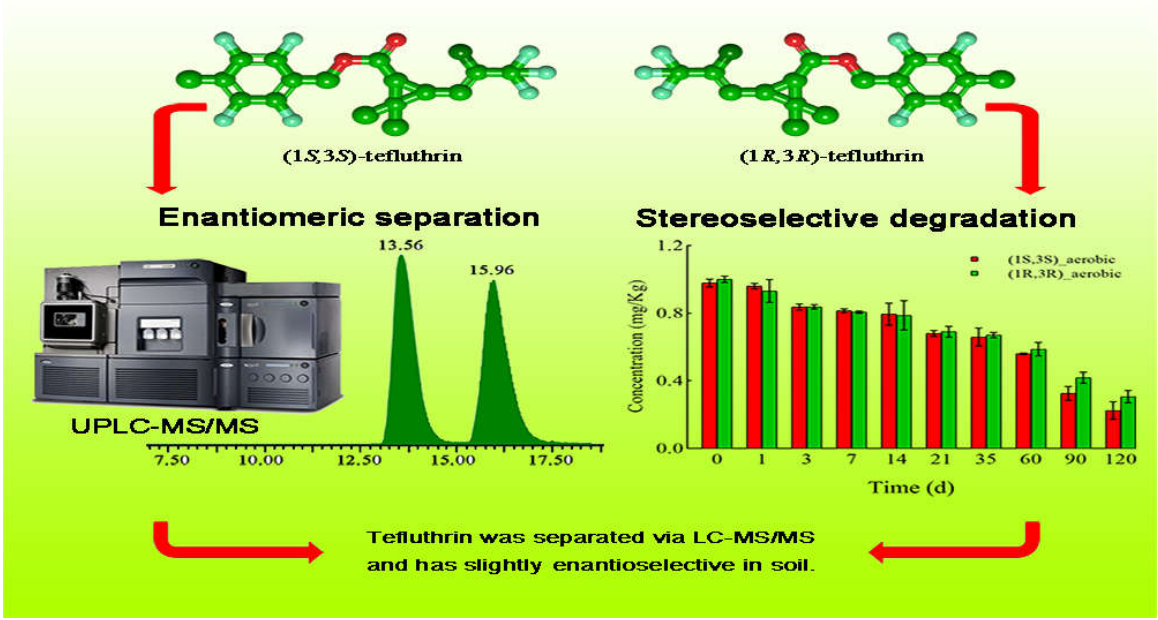
501

502 **Figure 3** Degradation of tefluthrin enantiomers in Nanjing soil. a. aerobic condition; b.

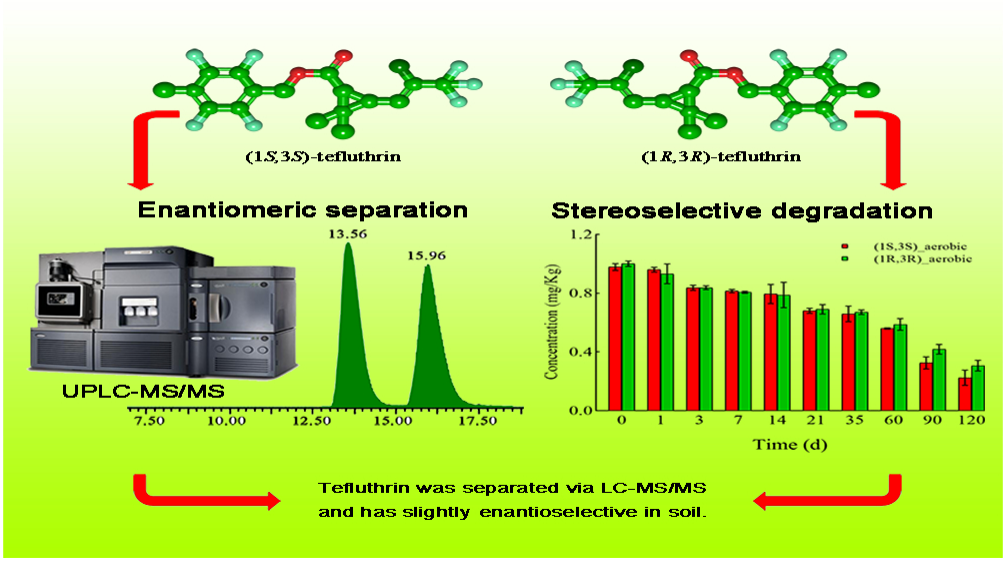
503 anaerobic condition; c. sterile condition; d. EF value

504

505 **TOC**


(1S,3S)-tefluthrin
(1R,3R)-tefluthrin
Enantiomeric separation
Stereoselective degradation
13.56
15.96
UPLC-MS/MS
7.50
10.00
12.50
15.00
17.50
1.2
0.8
0.4
0.0
Concentration (mg/Kg)
(1S,3S)_aerobic
(1R,3R)_aerobic
0
1
3
7
14
21
35
60
90
120
Time (d)
Tefluthrin was separated via LC-MS/MS
and has slightly enantioselective in soil.

506


(1S,3S)-tefluthrin
(1R,3R)-tefluthrin
Enantiomeric separation
Stereoselective degradation
13.56
15.96
UPLC-MS/MS
7.50
10.00
12.50
15.00
17.50
(1S,3S)_aerobic
(1R,3R)_aerobic
Concentration (mg/Kg)
1.2
0.8
0.4
0.0
0
1
3
7
14
21
35
60
90
120
Time (d)
Tefluthrin was separated via LC-MS/MS
and has slightly enantioselective in soil.