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Stereoselective dissipation of epoxiconazole in grape (*Vitis vinifera cv*. Kyoho) and soil under field conditions

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ABSTRACT

Stereoselective dissipation of epoxiconazole had been studied in grape and soil during plant growing under field conditions in this paper. A sensitive and rapid chiral method was developed and validated for the determination of epoxiconazole stereoisomers in grape and soil based on liquid chromatography coupled with triple quadrupole mass spectrometry (LC–MS/MS). Phenomenex Lux Cellulose-1 column was used for enantioseparation with a mixture of acetonitrile/water (90/10, v/v) as mobile phase at flow rate of 0.3 mL min^{-1}. Fortified recoveries in grape and soil samples ranged from 76.0% to 91.9% and relative standard deviations were less than 11.4% with fortified levels of 0.025–1.0 mg kg^{-1}. The limits of detection and quantification were 0.005 mg kg^{-1} and 0.025 mg kg^{-1}, respectively, with linear calibration curves extending up to 5.0 mg kg^{-1}. The field experimental results showed that dissipations of epoxiconazole stereoisomers in grape followed first-order kinetics ($R^2 > 0.92$) and stereoselectivity occurred in 2 h after spraying. The (−)-stereoisomer with half-life of 9.3 d degraded faster than (+)-stereoisomer with that of 13.2 d, and resulted in relative enrichment of (+)-stereoisomer. However, the stereoisomeric dissipations in soil were triphasic ("increase–decrease–steady") with lower dissipation rates, and also occurred with preferential degradation of (−)-stereoisomer under field condition. The results for stereoselective dissipations can be applied for food and environmental assessments of chiral pesticides.

1. Introduction

Most of currently used chiral pesticides are produced and released into the environment as racemates. In recent years, great attention has been focused on enantiomer-specific profiles of chiral contaminants. More and more studies have revealed that biological transformation of the chiral pollutants can be stereoselective, metabolism, uptake, excretion, environmental fate and ecological risk among enantiomers from one chiral contaminant were much different (Lewis et al., 1999; Ali et al., 2005; Diao et al., 2010; Xu et al., 2011). In these situations, the evaluation of behavior of chiral pesticides in organisms or in the environment based on the data obtained from racemates is not insufficient if stereoselective behaviors happened (Wang et al., 2007). Consequently, it is of great significance to develop enantiomeric analysis methods of chiral pesticides, investigate the different environmental behavior and toxicology of the individual enantiomer, and supply more accurate data for evaluating the environmental risk and food safety (Liu et al., 2008).

Epoxiconazole, cis-1-[[3-(2-chlorophenyl)-2-(4-fluorophenyl) oxiranyl]ethyl]-1H-1,2,4-triazole, is a triazole fungicide and firstly produced by BASF Corporation. It acts as an inhibitor of ergosterol biosynthesis, thereby interfering with fungal cell membrane synthesis. It is used as a broad-spectrum fungicide with preventive and curative action for control of diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes in cereals, sugar beet, peanuts, oilseed rape, apple and ornamentals (Bertelsen et al., 2001). It is highly effective at low application rates of 25–125 g of epoxiconazole active ingredients per hectare in one dose per season applied to the fields. There are two chiral carbons in its molecule and present two diastereoisomers with four stereoisomers. However, present commercial pesticide product of epoxiconazole just contains a pair of enantiomers with 2*R*,3*S*- and 2*S*,3*R*-configurations (Tomlin, 2000). The chemical structures and molecular weights (MWs) of four epoxiconazole stereoisomers are shown in Fig. 1.

HPLC with chiral stationary phases is the most commonly utilized technique in chiral separation. The stereoselective separations of epoxiconazole were achieved on cellulose tris-3,5-dimethylbenylcarbamate or microcrystalline cellulose triacetate stationary phase on reversed-phase HPLC (Hutta et al., 2002; Tian et al., 2007; Han et al., 2008). In recent years, the combination of HPLC with triple quadrupole mass spectrometry (MS/MS) for

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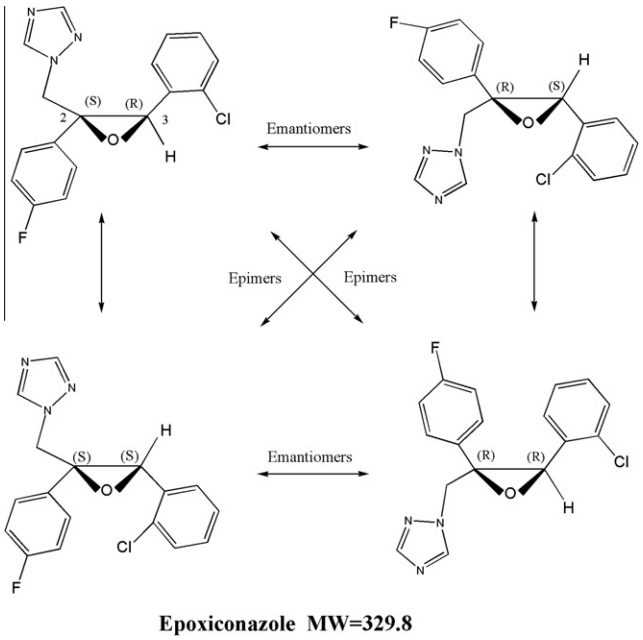

Fig. 1. Structures of epoxiconazole stereoisomers. The present commercial product refers to the *cis*-stereoisomers with 2*S*3*R* and 2*R*3*S* configuration. (Buerge et al., 2006).

analysis of pesticides have become increasingly popular due to its versatility, specificity and selectivity, enable the detection of target compounds in the low ng L^{-1} range (Gros et al., 2006), and be free of interferences from complex matrix samples such as vegetable, tissue and soil.

A number of reports have been published for determining residues and dissipation rate of racemic epoxiconazole in various samples (Zrostlíková et al., 2003; Schermerhorn et al., 2005; Trosken et al., 2005; Kovalczuk et al., 2006) and stereoselective degradation in soil. Bromilow et al. (1999a,b) studied degradation rates of five triazole fungicides in two soil types at fortification levels from 0.2 to 0.4 mg kg^{-1}, and concluded that under the given condition epoxiconazole was a very persistent pesticide with a half-life over 200 d. Buerge et al. (2006) investigated the influence of pH on the stereoselective degradation of the epoxiconazole in soils. However, few studies were involved in stereoselective environmental behaviors of epoxiconazole in grape and soil under field condition.

The present study is focused on investigation of possible stereoselective behaviors of epoxiconazole in grape and soil under the field condition, with a developed and validated chiral LC–MS/MS method for the analysis of epoxiconazole stereoisomers.

2. Materials and methods

2.1. Chemicals and reagents

Reference standards of *rac*-epoxiconazole (98.4%) were purchased from Shanghai Pesticide Research Institute (Shanghai, China). Stock solution of *rac*-epoxiconazole (500 mg L^{-1}) was prepared in acetonitrile and stored at −20 °C. Acetone, petroleum ether (60–90 °C), ethyl acetate and sodium chloride were of analytical grade and purchased from Beijing Chemical Reagent Co. Ltd. (Beijing, China). Petroleum ether was distilled before use. Acetonitrile was HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). Water was Wahaha pure water and purchased from Wahaha Group Co. Ltd. (Hangzhou, China). The Florisil solid phase extraction (SPE) cartridges (1000 mg/6 mL) were obtained from Agela Technologies (Tianjin, China) and conditioned with 5 mL petroleum ether before use.

2.2. Field trial

Field experiments were conducted on *Vitis vinifera cv*. Kyoho in Beijing (116.6 E, 39.8 N) of China in 2010, according to "Guidelines on Pesticide Residue Trials" (NY/T 788-2004) issued by the Ministry of Agriculture, the People's Republic of China. The plots with no application history of triazole fungicide group were selected, and any other fungicides with similar structure as that of epoxiconazole were forbidden to use in the period of the trial. The characteristic properties of the soil in the field were as follows: sandy clay loam; clay (%) $D < 0.002$ mm, 13.26; silt (%) $0.002 < D < 0.05$ mm, 31.57; sand (%) $0.05 < D < 2$ mm, 55.17; organic matter 12.3 g kg^{-1}; pH 8.24.

Each experimental treatment consisted of three replicate plots and a control plot without epoxiconazole. A 1-m distance was used as a buffer area to separate each plot. Four trial plots (three dissipation plots and one control plot) were prepared for grape dissipation experiments, and the area of each plot was 30 m^2. Epoxiconazole 30% SC (suspension concentrate, obtained from commercial source) dissolved in water was applied to three grape plots at 375 mg a.i. kg^{-1} (milligram of active ingredient in SC per kilogram). Another four plots with area of 10 m^2 each were prepared for soil dissipation study, epoxiconazole 30% SC dissolved in water was applied to three soil plots at 200 mg a.i. m^{-2}.

Representative samples were collected from each plot at different time intervals. The grape samples (at least 2 kg) were collected at 2 h, 1, 3, 5, 7, 10, 14, 21, 30 and 45 d after spraying. Soil samples (at least eight randomly selected sampling points for each plot) were collected at 2 h, 1, 3, 5, 7, 10, 14, 21, 30, 45 and 60 d after spraying with a soil auger. All the samples were mixed enough and stored at −20 °C until further analysis.

2.3. Sample preparation and extraction

The entire grapes were crushed thoroughly with a blender. A portion of 10-g minced sample was weighed into a 50-mL centrifuge tube, mixed with 20 mL of ethyl acetate by vortex for 1 min and ultrasonic for 20 min, and then centrifuged at 3000 r min^{-1} for 5 min. A 10-mL aliquot from the upper layer was evaporated to near dryness with a vacuum rotary evaporator at 30 °C, and the drying was completed with a nitrogen stream. The extract was redissolved in 5 mL of acetone/petroleum ether (3/7, v/v) before clean-up.

A portion of 10-g soil sample was weighed into a 50-mL centrifuge tube and mixed with 20 mL of acetonitrile by ultrasonic for 20 min. Sodium chloride (5 g) was subsequently added, and the tube was shaken vigorously by hand for 1 min and centrifuged at 3000 r min^{-1} for 5 min. Follow operations were same to those of grape sample.

2.4. Sample purification procedure

The concentrated extracts were transferred to florisil cartridge and eluted twice with 5 mL of acetone/petroleum ether (3/7, v/v). All eluates were collected and evaporated to near dryness with a vacuum rotary evaporator at 30 °C and to dryness with a gentle nitrogen stream. The residue was redissolved in 2 mL of acetonitrile and filtered through a 0.22-μm filter into a sample vial for LC–MS/MS analysis.