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Dissipation and Residues of Flutriafol in Wheat and Soil Under Field Conditions

Pingzhong Yu · Chunhong Jia · Wencheng Song · Fengmao Liu

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Abstract The dissipation and residues of flutriafol in wheat and soil under field conditions in Beijing, Anhui and Shandong in China were determined based on high performance liquid chromatography. Flutriafol were extracted with acetonitrile and cleaned up by solid-phase extraction with florisil cartridges before UV detection. The limits of detection and quantification of flutriafol were 0.04 ng and 0.01 mg/kg for both wheat and soil samples, respectively. The mean recoveries ranged from 93.4% to 96.4%, with relative standard deviation between 3.8% and 6.8% at three spiked levels (0.01, 0.1, 1.0 mg/kg). Half-lives were 13.3, 9.9 and 13.6 days in soil, while 15.2, 10.8 and 9.2 days in wheat plant in Beijing, Anhui and Shandong, respectively. The terminal residues of flutriafol were below the maximum residue limit 0.02 mg/kg set by Japan in wheat when pre-harvest interval were 35 days.

Keywords Flutriafol · Wheat · Soil · Dissipation

Flutriafol, (RS)-2,4′-difluoro-α-(1H-1,2,4-triazol-1-ylmethyl) benzhydryl alcohol (Fig. 1), is a triazole fungicide used in many crops for control of a broad spectrum of leaf and cereal ear diseases, particularly embryo borne diseases, e.g., bunts and smuts (Brown et al. 1986; Karaoglanidis et al. 2003). Its fungicidal mechanism of action is the inhibition of ergosterol biosynthesis and thus disruption of fungal cell wall synthesis.

Methods for the determination of flutriafol in various matrix were reported in recent years. In those methods, homogenized samples were extracted with acetonitrile/water (for plant materials) or acetonitrile (for animal commodities), and cleaned up with liquid–liquid partition followed by column chromatography using solid phase extraction (for plant materials) or gel permeation chromatography (for animal materials). Residues were determined by gas chromatography with nitrogen phosphorus detector or mass selective detector, or high performance liquid chromatography with MS/MS (Bolyg and Atreya 1991; Anagnostopoulos et al. 2012; Wang et al. 2010; Greulich and Alder 2008). These analytical methods for a range of substrates were validated with the low limits of quantification of the 0.01 mg/kg for flutriafol. However, to our knowledge, there was no study on the dissipation of flutriafol in wheat and its growing environment.

In this study, a simple and rapid method was developed for the extraction and cleanup of flutriafol from wheat and soil by high performance liquid chromatography (HPLC) first. The influence of environmental conditions on the dissipation and residue of flutriafol in wheat and soil under field conditions was discussed. These data will be helpful to establish the MRL of flutriafol in wheat and provide guidance on the proper and safe use of flutriafol.

Materials and Methods

Field trials were designed according to Guidelines on Pesticide Residue Field Trials (NY/T 788–2004, issued by

P. Yu · F. Liu (✉)
College of Science, China Agricultural University,
Beijing 100193, People's Republic of China
e-mail: liufengmao@yahoo.com

P. Yu · C. Jia
Institute of Plant Protection and Environmental Protection,
Beijing Academy of Agriculture and Forestry Sciences,
Beijing 100097, People's Republic of China

W. Song
Institute for the Control of Agrochemicals,
Ministry of Agriculture, Beijing 100125,
People's Republic of China

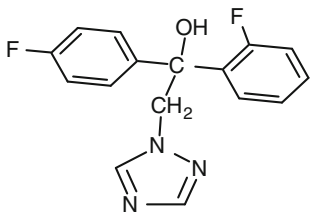

Fig. 1 Chemical structure of flutriafol

ministry of Agriculture, P. R. China), and experimental locations were at Fangshan District in Beijing, Hefei in Anhui Province, and Jinan in Shandong Province, China. The plot with no application history of flutriafol was selected. Each experimental treatment consisted of three replicate plots and a control plot. The control plots were separated by guard rows to avoid drifting contamination, etc. The area of each plot was 30 m^2. The trial was conducted from April to June in 2010. During the whole trial, the average minimum/maximum daily air temperatures were 11/33°C (Beijing), 15/35°C (Hefei) and 17/31°C (Jinan). The texture of soil is cinnamon soil (Beijing), yellow soil (Hefei) and alluvial soil (Jinan). To investigate the terminal residue, flutriafol (250 g/L, suspension concentrate) was applied at recommended dosage (90 g a.i/hm^2 with two treatments: spray 2 times and 3 times) with three replicate plots. To investigate the dissipation, 1.5 times of recommended dosage of flutriafol was applied at soil and straw with three replicate plots.

Soil samples were collected randomly from each plot using a soil auger to a depth of 0–10 cm. Plant samples were cut into small pieces and then ground with a mechanical slicer. Wheat samples were ground to powder. To investigate the dissipation of flutriafol, soil and plant samples were collected at 0 (2 h after application), 1, 3, 7, 14, 21, 28, 35, 42, 56 and 70 days after application. To investigate the terminal residue of flutriafol, wheat, straw and soil samples were collected at the harvest time. All samples were put into polyethylene bags and kept deep frozen (−20°C) until analysis.

In the dissipation study, the degradation percentage was calculated according to following formula. Dissipation rate (%) = ((C0–Ct)/C0) × 100, where, C0 referred to the fungicide concentration in primary treated soil, and Ct represented the fungicide concentration that remained in each dissipation stage.

Flutriafol standards (purity >99%) were purchased from J&K Scientific Ltd (Beijing, China). HPLC grade acetonitrile was purchased from Thermo Fisher Scientific (Waltham, USA). Hexane, acetone, and sodium chloride used in this study were analytical grade from Dikma (Beijing, China). A stock solution of 500 mg/L of flutriafol was prepared in acetonitrile. Working standard solutions (0.02, 0.05, 0.1, 0.5, 1, 2, 5 μg/mL), used for sample spiking and preparation of standard curve, were obtained from the stock solution by serial dilution with acetonitrile.

Ten grams of soil, straw and wheat samples were placed in a 250-mL conical flask and 100 mL acetonitrile and 5 g sodium chloride was added. The flask was capped and shaken for 1 h. The extracts were filtered with a filter paper, shaked vigorously, and set aside for 10 min. An aliquot of 50 mL upper layer were taken out to evaporate with a vacuum rotary evaporator at 40°C, and it was made to dryness under gentle nitrogen stream.

The florisil cartridge was preconditioned with 4 mL hexane. The concentrated extract was dissolved with 2 mL hexane and transferred into the florisil cartridge. The florisil cartridge was ringsed twice with 5 mL hexane–acetone (95:5, v/v), which was discarded. The florisil cartridge was then eluted with 5 mL hexane–acetone (80:20, v/v) three times sequentially, and the eluate was collected, concentrated to dryness, and dissolved in 2 mL acetonitrile for HPLC analysis.

All analyses were conducted with a Shimadzu 10ATvp HPLC equipped with ultraviolet detector (Shimadzu, Japan). ODS C18 HPLC column (250 × 4.6 mm i.d., 5 μm particle size) was maintained at 30°C. The mobile phase consisted of acetonitrile/water (50:50 v/v) with a flow rate of 0.8 mL/min. The injection volume was 10 μL, and the ultraviolet wavelength was 260 nm. The retention time of flutriafol was 3.8 min.

Quantification of flutriafol was accomplished using a standard curve prepared by diluting the stock solution in acetonitrile. Recovery tests were performed by spiking standards at the levels of 0.01, 0.1 and 1.0 mg/kg into samples with five replicates. The spiked and blank samples were prepared according to the method described above. Recovery was determined by comparing the amount of flutriafol recovered with the amount of fungicide added. The limits of detection (LODs) were defined as the concentrations of the flutriafol in wheat, straw and soil samples that gave a signal-to-noise (S/N) ratio of 3, and limits of quantification (LOQs) were just the lowest spiking levels in wheat, straw and soil.

Results and Discussion

The calibration graph obtained by plotting average peak area (each sample injected in duplicate) (y) versus concentration (x) was linear over the range of 0.02–5.0 μg/mL. The linear relation for flutriafol calibration could be expressed as a regression equation: $y = 25062\,x + 140.62$. Good linearity was obtained with a correlation coefficient of 0.9998.

The recovery and relative standard deviations (RSDs) of flutriafol from wheat, straw and soil samples spiked at 0.01, 0.1 and 1.0 mg/kg were listed in Table 1, and representative

chromatograms of standard and spiked samples were shown in Fig. 2. Recoveries of wheat, straw and soil samples ranged from 94.5% to 95.2%, while RSDs ranged from 3.8% to 6.8%. According to the Group of Analysts of Residues of Pesticides (1997), the acceptable values of variation coefficient are lower than 15%. Thus, the obtained values from extraction and cleanup procedure of this study are reliable for routine analysis of flutriafol in wheat and soil.

LODs were estimated to be 0.04 ng based on signal-to-noise ratio 3:1, and LOQs of flutriafol in wheat, straw and soil were 0.01 mg/kg. The LODs and LOQs data obtained in the present experimental conditions were all satisfactory, which were lower than MRLs of the investigated required by the regulation of the European Union (0.5 mg/kg) and Japan (0.02 mg/kg). These data confirm the availability of our method in detecting the flutriafol residues in samples.

Figure 3 summarized the dissipation of flutriafol in soil. The initial concentrations of flutriafol in soil were 0.61, 0.34 and 1.14 mg/kg, while half-lives were 13.3, 9.9 and 13.6 day in Beijing, Hefei and Jinan, respectively. The dissipation dynamics of flutriafol could be described by the following first order rate equation: $C = 0.3693\ e^{-0.0522t}$ (Beijing) with $r = 0.9743$, $C = 0.1952\ e^{-0.07t}$ (Hefei) with $r = 0.9525$, $C = 0.4603\ e^{-0.0511t}$ (Jinan) with $r = 0.9304$. These data showed the initial flutriafol deposits in soil differed among the three experimental sites. The dissipation of flutriafol in soil in Hefei was slightly faster than that in Beijing and Jinan. Additionally, the residues declined to 90% after 28 days in Hefei, but the residues declined to 90% after 35 days in Beijing and Jinan. No obvious difference was observed in dissipation rates between Beijing and Jinan, although soil properties and physical and chemical conditions, such as temperature and moisture, were varied. The persistence of pesticide in soil related to climate, soil properties and the physical and chemical properties of the pesticide (Pateiro-Moure 2008). In this study, not only soil properties but also the climate will cause the difference of dissipation rates in soil. Normally, the content of soil organic matter is positive correlation with soil microbial biomass (Insam and Domsch 1988). The highest level of organic matter content was found in the field trial location of Hefei. Hefei (33.38° north, 116.93° east) lies in the south of China and Beijing (39.6° north, 115.9° east) and Jinan (36.65° north, 117° east) lie in the north of China, the average temperature in the field trial period in Hefei was higher than in Beijing and Jinan. So it was predicted that the biomass population and the temperature may play a major effect role to cause the faster degradation.

Figure 4 summarized the dissipation of flutriafol in straw. The initial concentrations of flutriafol in straw were 1.02, 1.40 and 1.43 mg/kg in Beijing, Hefei and Jinan, respectively. The half-life time of flutriafol was 15.2, 10.8 and 9.2 days in Beijing, Hefei and Jinan, respectively. The dissipation dynamics of flutriafol could be described by the following equation: $C = 0.4979\ e^{-0.0456t}$ (Beijing) with $r = 0.9343$, $C = 1.2491\ e^{-0.0641t}$ (Hefei) with $r = 0.9645$, $C = 1.0654\ e^{-0.0851t}$ (Jinan) with $r = 0.9780$. From these results, it was evident that the initial flutriafol deposits in straw were no obvious difference among the three experimental sites, and the dissipation rate in straw was faster in Jinan than in Beijing and Hefei. Additionally, the residues declined to 90% after 28 days in Jinan, but the residues declined to 90% after 42 days in Beijing and Hefei. Usually, the dissipation of pesticides in the plant relates to physical and chemical factors like light, heat, pH and moisture (Dhananjay et al. 2005), rainfall during the field trial might have played a significant role. According to the field trial record, the rainfall in the field trial period was 482 mm in Jinan and was higher than in Beijing (139 mm) and Hefei (308 mm). This rain may have caused the different dissipation of flutriafol in straw.

Tables 2, 3 and 4 showed the terminal residue results. When flutriafol was applied at the recommended dosage over 2 or 3 times application, the residue levels of flutriafol in wheat in Beijing, Hefei and Jinan were <0.01–0.07 mg/kg,

Table 1 The fortified recovery of flutriafol in soil, straw and wheat sample (n = 5)

Sample	Spiking level (mg/kg)	Recovery					Average recovery (%)	RSD (%)
		1	2	3	4	5		
Soil	0.01	89.6	98.0	96.4	99.9	92.3	95.2	4.4
	0.1	93.5	88.8	96.4	98.3	95.4	94.5	3.8
	1.0	95.3	95.6	90.8	98.6	101.5	96.4	4.2
Straw	0.01	96.1	95.3	94.6	99.7	87.2	94.6	4.8
	0.1	102.2	94.6	85.5	91.1	96.6	94.0	6.6
	1.0	98.7	99.3	92.5	91.3	98.8	96.1	4.0
Wheat	0.01	97.3	90.1	89.9	96.4	96.3	94.0	3.9
	0.1	96.8	97.3	102.4	92.1	85.3	94.8	6.8
	1.0	87.7	92.3	97.9	91.8	97.3	93.4	4.5

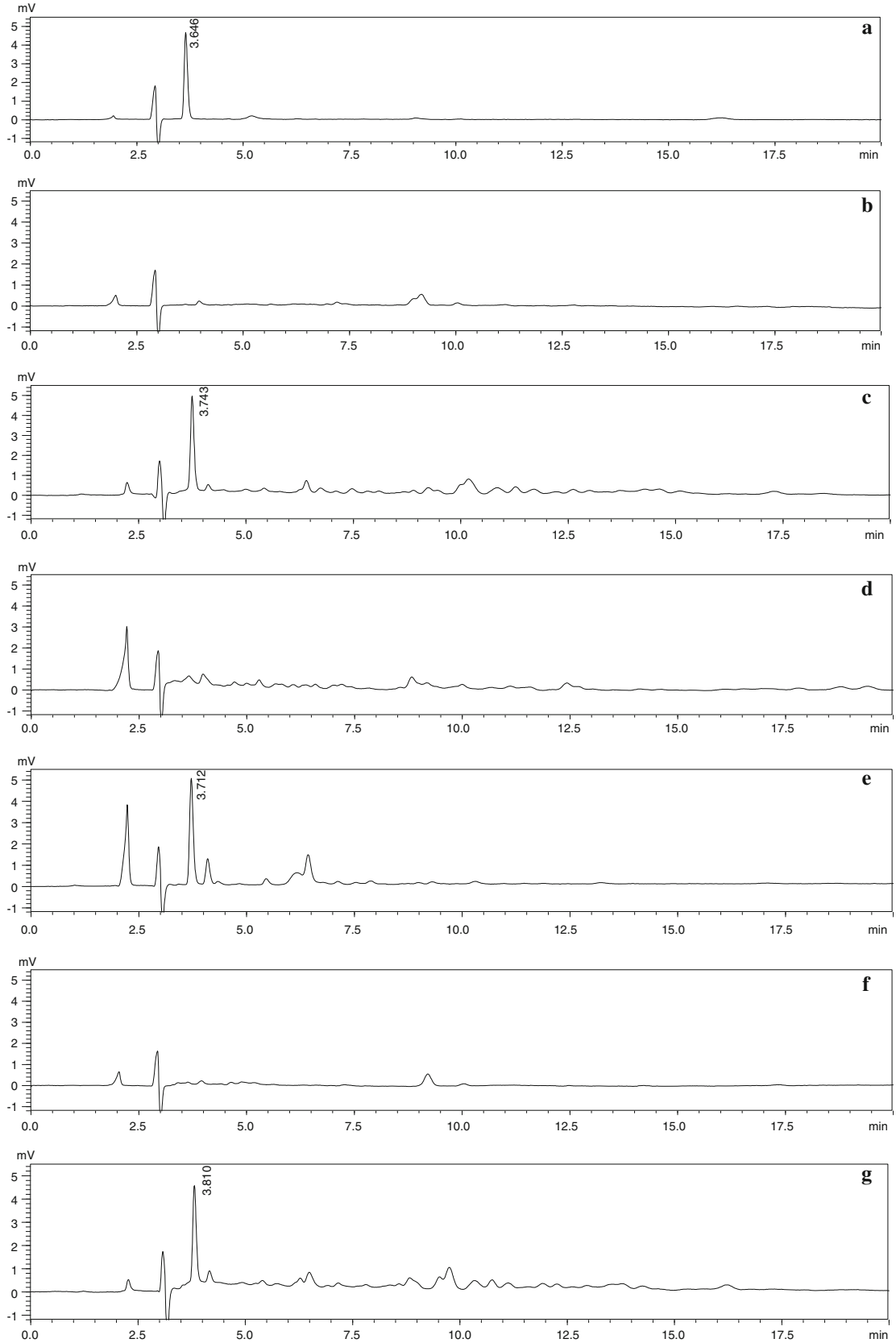

Fig. 2 Chromatograms for: **a** Flutriafol standard solution, **b** Blank soil sample, **c** Soil spiked at 0.1 mg/kg, **d** Blank plant sample, **e** Plant sample spiked at 0.1 mg/kg, **f** Blank wheat sample, **g** Wheat spiked at 0.1 mg/kg

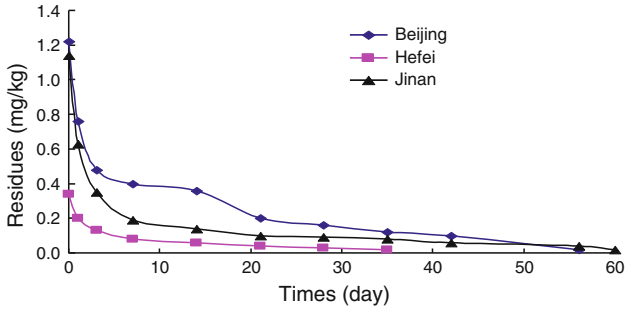

Fig. 3 Dissipation curves residues of flutriafol in soil in Beijing, Hefei and Jinan

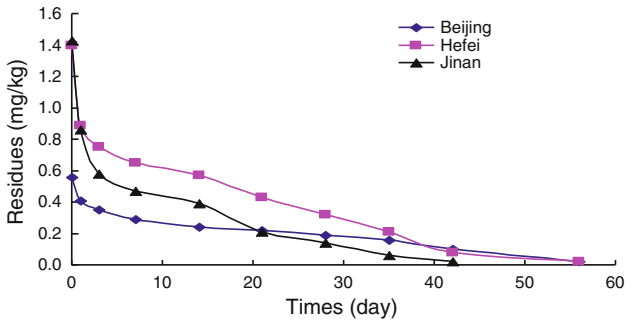

Fig. 4 Dissipation curves residues of flutriafol in straw in Beijing, Hefei and Jinan

Table 2 Terminal residues of flutriafol in wheat in Beijing, Hefei and Jinan, China

Dosage (g a.i./ha)	Application	PHI (d)	Residues (mg/kg)		
			Beijing	Hefei	Jinan
CK	/	/	<0.01	<0.01	<0.01
90	2	21	<0.01	<0.01	<0.01
		28	<0.01	<0.01	<0.01
		35	<0.01	<0.01	<0.01
	3	21	0.07	0.07	0.05
		28	0.04	<0.01	0.02
		35	<0.01	<0.01	<0.01

<0.01–0.07 mg/kg and <0.01–0.05 mg/kg, in straw were <0.01–0.11 mg/kg, <0.01–0.12 mg/kg and <0.01–0.03 mg/kg, in soil <0.01–0.16 mg/kg, <0.01–0.14 mg/kg and <0.01–0.10 mg/kg, respectively. China and Codex Alimentarius Commission (CAC) have not established maximum residue limits (MRLs) for flutriafol in wheat, while European Union (EU) and Japan set MRLs for flutriafol are 0.5 and 0.02 mg/kg, respectively. According to the terminal residue results, the highest residue in wheat at interval of 35 days was below 0.01 mg/kg. This result suggests that it is safe to harvest 35 days after applying the recommended dose of flutriafol.

Table 3 Terminal residues of flutriafol in straw in Beijing, Hefei and Jinan, China

Dosage (g a.i./ha)	Application	PHI (d)	Residues (mg/kg)		
			Beijing	Hefei	Jinan
CK	/	/	<0.01	<0.01	<0.01
90	2	21	<0.01	<0.01	<0.01
		28	<0.01	<0.01	<0.01
		35	<0.01	<0.01	<0.01
	3	21	0.11	0.06	0.03
		28	0.03	0.12	0.03
		35	<0.01	<0.01	<0.01

Table 4 Terminal residues of flutriafol in soil in Beijing, Hefei and Jinan, China

Dosage (g a.i./ha)	Application	PHI (d)	Residues (mg/kg)		
			Beijing	Hefei	Jinan
CK	/	/	<0.01	<0.01	<0.01
90	2	21	<0.01	<0.01	<0.01
		28	<0.01	<0.01	<0.01
		35	<0.01	<0.01	<0.01
	3	21	0.16	0.14	0.10
		28	0.07	0.04	0.06
		35	0.02	0.02	<0.01

To sum up, the dissipation and residues of flutriafol in wheat and soil under field conditions of in China was investigated. This work would be useful in the establishment of a maximum residue limits and the safe and proper use of flutriafol in wheat in China.

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