Bull Environ Contam Toxicol (2008) 81:360–364
DOI 10.1007/s00128-008-9470-1

Analytical Method for Estimation of a New Insecticide Flubendiamide and its Safety Evaluation for Usage in Rice Crop

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Received: 6 October 2007 / Accepted: 14 May 2008 / Published online: 28 June 2008

Abstract The paper presents a method for residue analysis of flubendiamide in rice (*Oryza sativa*), which includes improved extraction, cleanup and determination of flubendiamide in rice seeds, husk and straw by using LC with UV detection. Safety evaluation of this insecticide in rice has been carried out after applying its soluble concentrate (SC) formulation at recommended dose (30 g a.i. ha^{-1}) and double of the recommended dose (60 g a.i. ha^{-1}) on rice crop. MRL of flubendiamide on rice grain can be proposed as 0.2 mg kg^{-1}.

Keywords Flubendiamide · Rice (*Oryza sativa*) · Residue analysis · MRL

Flubendiamide is NNI-0001, N^2-(1,1-dimethyl-2-methyl sulphonyl ethyl)-3-iodo-N^1-[2-methyl-4-{1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl} phenyl] 1,2–benzene dicarboxamide (Fig. 1). It belongs to phthalic acid diamide (Diaz de Toranzo and Brieux 1967) insecticide developed by Bayer Crop Science, Germany in collaboration with Nihon Nohyaku Co. Ltd., Tokyo, Japan. The uniqueness of the structure results from three parts with novel constituents: a heptafluoroisopropyl group in the anilide moiety, a sulfonylalkyl group in the aliphatic amide moiety, and an iodine atom at the 3-position of the phthalic acid moiety. It is mainly effective for controlling lepidopteron pests including resistant strain in rice, cotton, corn, grapes, other fruits and vegetables (Pessah 1989; Tohnishi et al. 2005a). It has larvicidal activity as a stomach poison and is an oral intoxicant, fast acting (rapid cessation of feeding), long lasting (rain fast) and has limited plant penetration and systemicity. It has a novel biochemical action as it affects calcium ion balance irrespective of sodium or potassium ion balance (Hall et al. 1995), which causes contraction of insect skeletal muscle (Usherwood 1962).

Flubendiamide has been used in plant protection as a new agricultural material for productivity improvement demonstration test and for noxious insect prevention measure test with chemical pest control in Japan (Tohnishi et al. 2005b). The persistence study of flubendiamide and its metabolite NNI-0001-des-iodo; N^2-(1,1-dimethyl-2-methylsulfonyl-ethyl)-N^1-[2-methyl-4-{1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl} phenyl]-1,2-dicarboxamide, was carried out in soil by Bayer laboratories and they recommended the use of Liquid chromatography/mass spectroscopy (LC/MS) for their detection (Bayer 2003). LC/MS is costly and is not commonly available in Indian Laboratories as well as in other developing countries. However, there is no report of a suitable methodology for the determination of flubendiamide residue in crops.

This paper presents a method for residue analysis of flubendiamide in rice (*Oryza sativa*), which includes extraction, cleanup and determination of flubendiamide in rice seeds, husk and straw by using High Pressure Liquid Chromatography (HPLC) with UV detection and proposes its MRL in rice besides evaluating safety of this insecticide.

Materials and Methods

Acetone, dichloromethane and hexane were purchased locally and distilled before use. A 50.761 mg portion of

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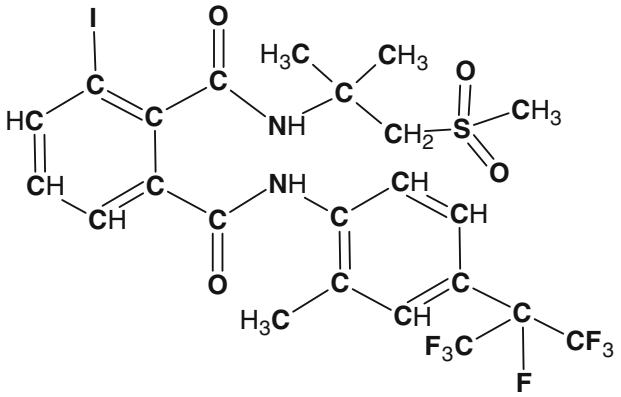

Fig. 1 Chemical structure of flubendiamide, a phthalic acid diamide

analytical grade flubendiamide (purity 98.5%), supplied gratis by Bayer Crop Science GmbH, Germany, was dissolved in 50 mL HPLC-grade acetonitrile to obtain a 1,000 μg mL^{-1} stock solution. Solutions of various concentrations were prepared by diluting this standard solution. Mixture of acetonitrile and water was used as a mobile phase with a flow rate of 0.5 mL min^{-1}. The injection volume was 10 μL and the wavelength was set at 210 nm in HPLC system-LaChrom L-7100 dual pump, L-7400 UV detector, L-7200 autosampler Hitachi High Technologies (San Jose, CA). Concentrations versus peak area obtained on analyzing standard solutions was plotted for the determination of flubendiamide present in test samples.

A 25 g portion of crushed rice grains was fortified with flubendiamide at 2 and 4 μg g^{-1} separately in triplicate. Each fortified sample was extracted with 100 mL, 1:1 hexane:acetone solvent mixture in a shaker for 4 h and the extract was filtered under suction. The extract was then evaporated by using a rotary vacuum evaporator. Each fortified sample was transferred to a separatory funnel, and 150 mL aqueous solution (2%, wv^{-1}) of sodium chloride was added. The aqueous phase was partitioned three times with 30 mL portions of hexane and passed through anhydrous sodium sulphate. The combined organic layers were evaporated to dryness and reconstituted in 10 mL of hexane prior to column cleanup.

The residues obtained after concentration was subjected to column cleanup with different adsorbents to optimize the cleaning procedure. Chromatographic columns (glass, 60 × 1.5 cm id) were packed with different adsorbents. Activated anhydrous sodium sulfate (2 g) + neutral alumina (5 g) + activated anhydrous sodium sulfate (2 g) combination was chosen for cleanup. Each column was prewashed with 30 mL hexane. The pesticide concentrate obtained above was dissolved in 10 mL hexane and transferred to the column. It was then allowed to adsorb on the column. The column was eluted with (a) 50 mL hexane followed by (b) 100 mL hexane–acetone (9:1 vv^{-1}), then these two fractions were discarded and finally the column was eluted with (c) 100 mL hexane:acetone (7:3 vv^{-1}). The final elute from each column was concentrated to dryness by using a rotary evaporator. The residue was dissolved in 10 mL HPLC-grade acetonitrile before analysis. Each cleanup was performed in triplicate with a control for each set (Table 1). While Limit of detection (LOD) was 0.01 mg kg^{-1}, Limit of quantification (LOQ) was 0.025 mg kg^{-1} for the analysis of these samples.

A 25 g portion of rice husk (the outer non edible portion) was fortified with flubendiamide at 2 and 4 μg g^{-1} in triplicate along with one control to which flubendiamide was not added. Each sample including control was then extracted with 300 mL hexane:acetone (95:5 vv^{-1}) in Soxhlet for 4 h. The extract obtained was then evaporated to dryness by using rotary evaporator. The residue was transferred to a separatory funnel and 150 mL sodium chloride solution (2%, wv^{-1}) was added. The aqueous phase was partitioned with 30 mL portions of hexane three times. The organic layer was then evaporated to dryness. The residue obtained after concentration was subjected to column cleanup. Chromatographic column was packed with anhydrous sodium sulfate (2 g) + activated silica gel (60–120 mesh; 5 g) + anhydrous sodium sulfate (2 g). The column was then pre-washed with 30 mL hexane. The pesticide concentrate obtained above was loaded on the column by dissolving in 5 mL hexane and eluted with (a) 50 mL hexane followed by (b) 150 mL hexane:acetone (9:1) and (c) 100 mL hexane:acetone (8:2). First and second fractions of column were discarded and third (i.e.) (c) fraction was concentrated to dryness by using rotary vacuum evaporator. The residue was dissolved in 10 mL LC-grade acetonitrile before analysis (Table 2).

Portions (50 g) of chopped rice straw were fortified with flubendiamide at 2 and 4 μg g^{-1} separately in triplicate. No insecticide was added in control sample of rice straw. The flubendiamide residues were extracted and cleaned up by following the procedure described for rice husk.

Results and Discussion

During method development the average recoveries of flubendiamide from rice seed, husk and straw ranged from 79.1% to 90.5% (for husk) at 2 and 4 μg g^{-1} (Table 1 & 2). HPLC with UV detection revealed that the choice of mobile phase is crucial for the separation of the parent pesticide from co extractives. Mobile phase consisting of acetonitrile:water (70:30 vv^{-1}) was found to give optimum resolution with a suitable retention time of 10.53 min (Fig. 2). The use of hexane:acetone (1:1) for extraction of flubendiamide from rice seed gave satisfactory recoveries

Table 1 Recovery of flubendiamide from rice grains

Solvent system used hexane:acetone	Amount added (μg)	Amount recovered (μg)			Mean (±SD) (μg)	Recovery (%)
		R1	R2	R3		
Hexane	4	–	–	–	–	–
	2	–	–	–	–	–
9:1	4	–	–	–	–	–
	2	–	–	–	–	–
7:3	4	3.478	3.142	3.130	3.250 (0.1975)	81.27
	2	1.423	1.221	1.202	1.282 (0.1224)	79.13

–: Not detected (<0.025 μg g^{-1})
SD = Standard deviation

Table 2 Recovery of flubendiamide from rice husk

Solvent system used hexane:acetone	Amount added (μg)	Amount recovered (μg)			Mean (± SD) (μg)	Recovery (%)
		R1	R2	R3		
9:1	4	–	–	–	–	–
	2	–	–	–	–	–
8:2	4	3.423	3.925	3.515	3.621 (0.2673)	90.54
	2	1.621	1.843	1.930	1.798 (0.1593)	89.92

as cold extraction method was adopted for rice grain. On the other hand, hot extraction with hexane:acetone (95:5) was used for extraction of flubendiamide residues from rice husk. In order to minimize the amount of interfering co-extractives during hot extraction, the polarity of solvent combination was reduced by increasing the ratio of hexane. Due to cold extraction, co-extractives were less and the recovery of the compound was still respectable. Different solvents of various polarities were used for liquid–liquid partitioning to extract flubendiamide residues from aqueous phase. In the case of partitioning with dichloromethane, a peak due to some co-extractive was observed at the same retention time as that of flubendiamide (10.53 min) during HPLC analysis. So it interfered in the analysis of residue of flubendiamide in treated samples. To overcome this problem, partitioning was done with hexane. The average recoveries obtained on using hexane were 81.3% in the case of grains and 90.5% in the case of husk and straw, when these were spiked at 0.5 and 1 μg g^{-1} (Table 3).

Flubendiamide (SC 480 Formulation) was applied at recommended dose (62.5 mL ha^{-1} formulation; 30 g a.i. ha^{-1}) and double of recommended dose (125 mL ha^{-1} formulation; 60 g a.i. ha^{-1}) on rice crop. The method developed for the analysis of residue of flubendiamide in laboratory was applied for the analysis of samples generated from field trials. Treated samples were analyzed for the residues present at the time of harvest in field trials.

The insecticide formulation was applied at the recommended rate and double the recommended rate in separate plots; control plots were not sprayed with the pesticide (Figs. 3 and 4). In the treated samples of rice, rice grains were found to contain maximum residue i.e. 0.823 μg g^{-1} from the plot treated with double dose. However, residues

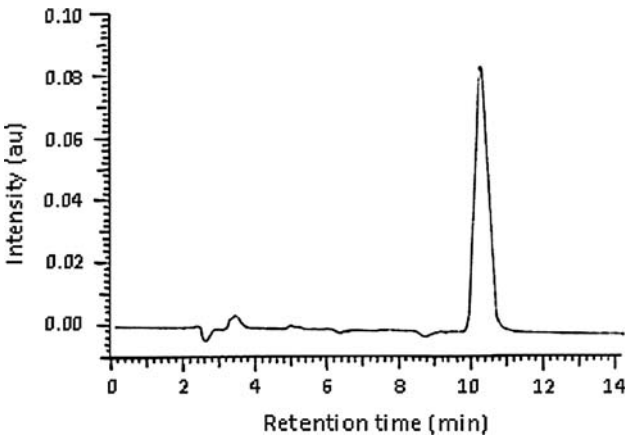

Fig. 2 HPLC chromatogram of flubendiamide in acetonitrile:water (70:30)

were below detectable limit (0.025 μg g^{-1}) in husk and straw collected from the plots treated with recommended dose of the insecticide (Table 4). One major metabolite of flubendiamide namely NNI-0001-des-iodo, which is formed in soil during photolysis (Bayer 2003) was also monitored.

Its retention time was 8.66 min when HPLC analysis was done under the same condition in which flubendiamide was analyzed. This toxic metabolite was not found in edible commodity analyzed at the time of harvest. Only the grains of rice are consumed by human being therefore the level of pesticide recorded in/on them were used for proposing the MRL. Residue implication of foliar application of flubendiamide on rice crop has been evaluated by comparing "Theoretical Maximum Residue Contribution (TMRC)" of the pesticide with its "Maximum Permissible

Table 3 Recovery of flubendiamide from rice grains, husk and straw from field samples

Commodity	Pesticide added (μg/g)	Pesticide recovered			Mean (±SD)	Recovery (%)
		R1	R2	R3		
Grain	0.5	0.389	0.405	0.430	0.408 (0.0169)	81.63
	1	0.807	0.794	0.817	0.806 (0.0094)	80.56
Husk & Straw	0.5	0.451	0.460	0.436	0.449 (0.0228)	89.87
	1	0.923	0.898	0.915	0.912 (0.0540)	91.23

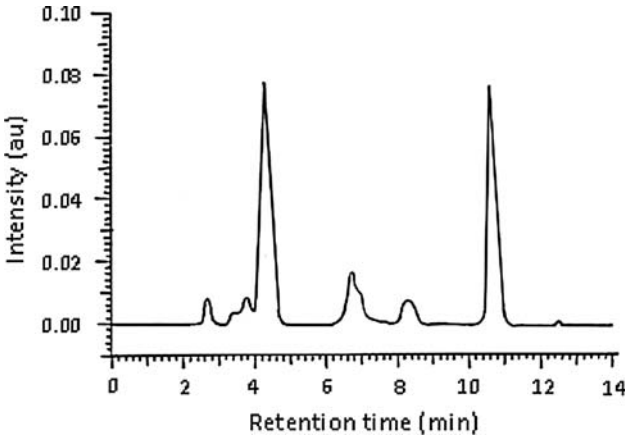

Fig. 3 HPLC chromatogram of insecticide spiked sample of rice grain

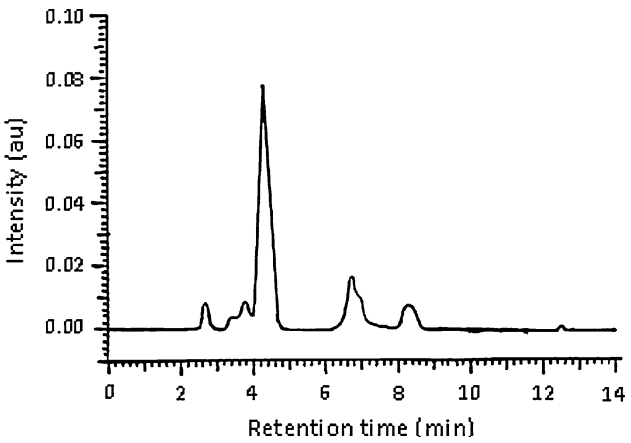

Fig. 4 HPLC chromatogram of control samples of rice grain

Intake (MPI)" (Gopal and Mukherjee 1995). TMRC was calculated by assuming that all the cereals utilized would contain the insecticide residue at the highest level found in this study following the application of flubendiamade. The recommended balanced diet for an adult Indian according to Indian Council of Medical Research (ICMR) states that the per capita consumption of cereals in India is 675 g day^{-1}, out of 1.5 kg food consumed per day, i.e. 45% can be taken as food factor for cereals. Hence, the

Table 4 Residues found in field samples of rice treated with recommended and double dose of flubendiamide

Commodity	Pesticide dose	R1	R2	R3	Average
Rice grain	Recommended	0.025	0.111	ND	0.043
	Double	0.971	0.768	0.732	0.823
Rice husk	Recommended	ND	ND	ND	ND
	Double	ND	ND	ND	ND
Rice straw	Recommended	ND	ND	ND	ND
	Double	ND	ND	ND	ND

ND, Non detectable (<0.025 μg g^{-1})

TMRC was found by multiplying maximum residue level obtained during recommended dose of application with food factor and total intake of food per day.

$$\text{TMRC} = 0.111\ \text{mg kg}^{-1} \times 45\% \times 1.5\ \text{kg} = 0.074925\ \text{mg person}^{-1}\ \text{day}^{-1}$$

MPI was obtained by multiplying ADI (Acceptable Daily Intake) with average body weight of an adult taken as 65 kg. On the other hand, the ADI was calculated by dividing Non-Observed-Adverse-Effect-Level (NOAEL) of flubendiamide (2.6 mg kg^{-1}) with safety factor, which was taken as 100.

$$\text{ADI} = 2.6/100 = 0.026\ \text{mg kg}^{-1};\ \text{MPI} = 0.026 \times 65 = 1.69\ \text{mg person}^{-1}\text{day}^{-1}$$

Here the TMRC of flubendiamide (Maximum value 0.0749 mg $person^{-1}$ day^{-1}) is less than its MPI (1.69 mg $person^{-1}$ day^{-1}), this proves that the schedule is safe. The highest residue found among the replicates (n = 3) was 0.111 mg kg^{-1}. Therefore considering the convention of Codex Alimentarius Commission (1986) MRL of this insecticide on rice grain can be proposed as 0.2 mg kg^{-1}. The value is in accordance with the practice of the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) of expressing MRL only in units of 1, 2, 5 and 10. More multilocational trials will however be required for establishing MRL. This data when combined with residues data generated from other countries of the world will be useful for Codex Alimentarius Commission to fill a gap in information.

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