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Simple and Rapid Method for Determination of Flubendiamide and its Residues in Soil and Water by HPLC-UV Detection

M. Paramasivam[ab]* and Hemanta Banerjee[a]

[a]AINP on Pesticide Residue Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidhyalaya, Kalyani -741 235, West Bengal, India.

[b]Present address: Pesticide Toxicology Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu.

A simple and rapid method for the analysis of flubendiamide and its major metabolite desiodo flubendiamide, in soils and water with acetonitrile and ethyl acetate, respectively, is presented. Quantification was performed by reversed phase HPLC with UV detector. Mean recoveries of flubendiamide and desiodo flubendiamide in soils ranged from 88.01- 90.26 % and 90.5-92.29 % respectively. The applicability of the method to the determination of flubendiamide and desiodo flubendiamide in spiked water at three pH levels (4, 7 and 9.2) was evaluated. Recoveries for flubendiamide and desiodo flubendiamide spiked water were in the ranges 89.21-92.32 % and 92.2-95.57 % respectively. The detector linearity and the repeatability of the method were very precise.

Key words: Flubendiamide, Desiodoflubendiamide, HPLC-UV, Soil, Water.

*[ab]Corresponding author email: sivam25@gmail.com

Flubendiamide: N2-(1,1-dimethyl-2-methyl sulphonyl ethyl)-3-iodo-N1-[2-methyl-4-{1,2,2,2-tetrafluoro- 1-(trifluoromethyl) ethyl} phenyl] 1,2–benzene dicarboxamide and des-iodo flu bendiamide: N2-(1,1-dimethyl-2-methyl sulfonylethyl) - N1-[2-methyl-4-{1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl} phenyl]-1,2-dicarboxamide (Figure 1), is a novel insecticide belonging to class benzene dicarboxamide (Ebbinghaus *et al.,*2006), ryanodine receptor modulator type of biochemical action and mainly effective for controlling lepidoptera pests including resistant strains in rice, cotton, corn, grapes, other fruits and vegetables and gram pod borer (Tohnishi *et al*., 2005; Masaki *et al*.,2006; Ohkawa *et al*., 2007). It is hydrolytically stable, relatively immobile in soils, practically non-detectable in key rotated crops, mobile in the xylem following penetration into plant tissue, and exhibits strong rain fast characteristics due to the unique chemical properties (Ohkawa *et al*., 2007; Shane *et al*., 2006).

Few analytical methods have been reported for determination of this compound in soil (Shane *et al*., 2006; Mohapatra *et al*., 2010; Sahoo *et al*., 2009). However, there are no reports on the analysis of flubendiamide and its metabolite desiodo flubendiamide from water samples. Determination of flubendiamide and its metabolite desiodo flubendiamide residues in soil and water is necessary so that harmful effects of the pesticide minimized. The study pertaining to its persistence, behaviour in soil and water is essential to get an

idea about the extent of residues formation thereby predicting the risk of residual toxicity and ground water pollution. In the present work, a simple, rapid, inexpensive, and eûcient method to simultaneously determine flubendiamide and its major metabolite desiodo flubendiamide in different soils and water at different pH levels is presented.

Materials and Methods

Analytical reference standards of flubendiamide (98.5% purity) and desiodo flubendiamide (99.3 % purity) were obtained from M/s Rallis India Limited Bangalore, India. Organic solvents like acetonitrile, ethyl acetate, dichloromethane and hexane were glass distilled before use. Sodium sulfate was washed with acetone and activated at 110 ºC for 4 h before use. HPLC grade solvents were procured from M/s Merck India Ltd. These were filtered and de-gassed prior to use.

Individual stock solutions (1000 µg mL^{-1}) of the analytical standard were prepared by dissolving 100 mg of each compound in 100 mL of acetonitrile and were stored at 4 ºC. Working standard solutions were prepared by appropriate serial dilutions in acetonitrile for the fortification of soil and water samples and the construction of calibration curves, respectively. All working standard solutions were kept under refrigerated conditions (4-5 ºC).

Collection and preparation of soil samples

The soils of three different agro-climatic zones of West Bengal: New alluvial zone (Mondouri), Red and lateritic zone (Jhargram) and Coastal zone

(Canning) were collected (1 kg) at random from 6-8 places at a depth of 0-15 cm having no previous history of flubendiamide application. Soils were air dried, ground and passed through a 2.0 mm sieve and sub-sampled by quartering.

Extraction and cleanup procedure

The fortified soil sample (20 g each) was shaken for half an hour in a mechanical shaker with 100 mL acetonitrile in a conical flask and the extract was filtered through filter paper (Whatman No.42) mounted on a buchner funnel with 100 mL of acetonitrile. The pooled filtrate was transferred into a 500 mL round bottom flask and concentrated to about 50 mL using a rotary evaporator in a water bath at 40º C.

The concentrated acetonitrile extract was transferred quantitatively to a 500 mL separating funnel. The sample was partitioned thrice with 100 mL hexane (saturated with acetonitrile) and the upper hexane layer was discarded each time. The lower acetonitrile layer was partitioned against dichloromethane (3 x 100 mL) by addition of 4 % saturated NaCl solution. The combined organic layer was dried over anhydrous sodium sulphate and collected into a 500 mL pear shaped flask and concentrated to dryness on a rotary evaporator (40 ºC). The residues were reconstituted in mobile phase, ûltered through 0.45 µm membrane ûlters and transferred to autosampler vials for the instrumental analysis in the HPLC-UV system.

Preparation of water sample

Water samples of different pH were prepared by using the buffer powder (Rankem) pH = 4.0, pH = 7.0 and pH = 9.2) by dissolving each powder packet in 100 mL distilled water. The pH was verified by pH meter.

Extraction and cleanup

The fortified water samples (100 mL) at different pH viz., 4.0, 7.0 and 9.2 were transferred separately into separating funnels of 500 mL capacity which were saturated with addition of 50 mL NaCl solution (4%) and partitioned with 100 mL distilled 40% ethyl acetate in hexane (3x100 mL). The upper organic phase was collected in pear shaped flask by passing over anhydrous Na_2SO_4 and evaporated to dryness in rotary vacuum evaporator (40 ºC). The residues were reconstituted with acetonitrile: water (60:40, v/v), filtered through 0.45 µm membrane filters and transferred to autosampler vials for HPLC analysis.

Determination of Recovery

Fortification of soil samples

A representative samples (20g each) of different soil samples viz. new alluvial, red and lateritic and coastal soil were transferred in 250 mL Erlenmeyer flask. Soil samples were fortified with working standard solution to furnish concentrations of 0.01, 0.05, and 0.1 µg g^{-1}. After spiking, samples were allowed for 30 minutes at ambient temperature to evaporate the acetonitrile, and then processed. All recovery experiments were conducted in replicates of three (n = 3). The control samples were processed by a similar stepwise procedure to check for interference from the matrix.

Fortification of water samples

Recovery study was carried out in order to establish the efficiency and reliability of the analytical method employed. Distilled water samples, maintained at pH 4.0, 7.0 and 9.2 levels, were fortified at the level of 0.01, 0.05 and 0.1 µg mL^{-1} with the test mixture of analytical standard solution and analyzed in HPLC. The control samples were processed by a similar stepwise procedure to check for interference from the matrix.

Liquid-chromatographic determination

The analysis of flubendiamide and desiodo flubendiamide in soil and water using reverse phase HPLC technique was used for quantitative analysis.

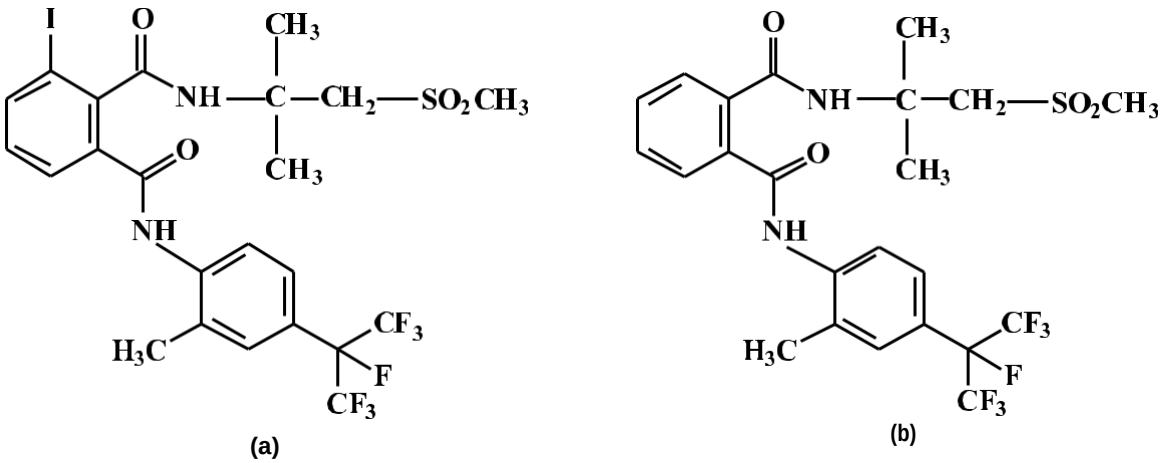

Figure 1. Chemical structures of (a) flubendiamide, and (b) desiodo flubendiamide

Agilent 1200 series with chemstation software, C-18 column, BDS Hypersil, 25cm length x 4.6 mm i.d. and 0.5mu particle size, Mobile phase (acetonitrile:water (60:40,v/v) at 1 mL flow rate and detector set at 210 nm ëmax was used for analysis of flubendiamide and desiodo flubendiamide showed sharp peak at 9.77 and 7.66 minutes respectively (Figure 2) under the described HPLC condition. The quantification was done using external working standard calibration curves.

Results and discussion

Linearity

Under the chromatographic conditions described above, calibration graphs were constructed by plotting peak area versus concentration, and good linearity was achieved in the range of 0.01- 1.0 µg mL^{-1}. The correlation coefficients derived from linear regressions were in both cases higher than 0.99989 which signified the

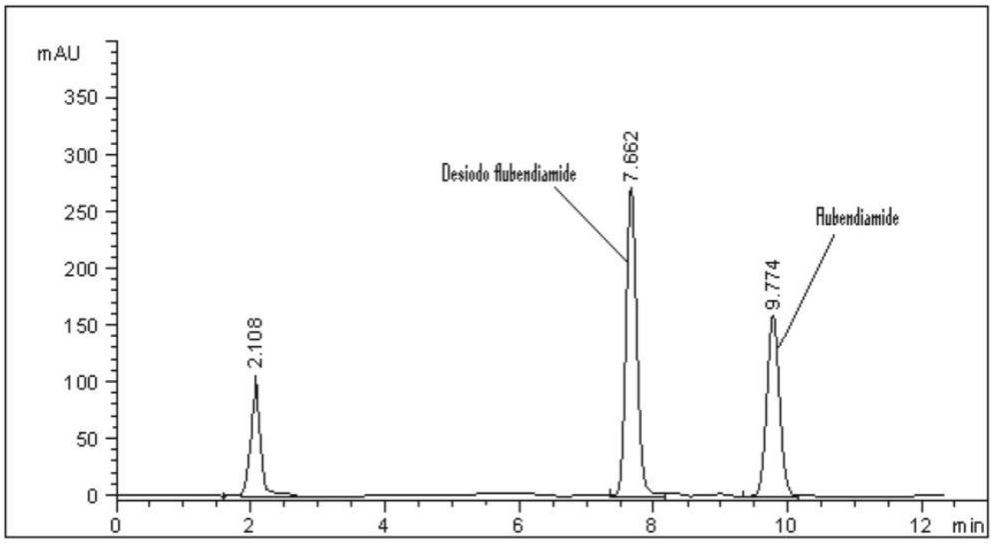

Figure 2. HPLC-UV chromatograms of flubendiamide and desiodo flubendiamide (0.1 µg g^{-1})

optimization of HPLC. Retention time and peak area were checked for repeatability by injecting test mixture standard solution into the HPLC system over three runs.

Sensitivity

The limit of quantification (LOQ) of this method, defined as the lowest concentration of compounds in a sample that could be quantitatively determined with suitable precision and accuracy, was 0.01 µg g^{-1}. The limits of detection (LOD) were established by considering a value of three times the background noise of the blank sample at the retention time of each pesticide. The limit of detection (LOD), determined as the concentration for which peak heights were three times the baseline noise, were 0. 003, µg g^{-1} for flubendiamide and desiodo flubendiamide.

Recovery study

The new analytical method was developed and validated for the determination of flubendiamide and

Table 1. Recoveries and RSD of flubendiamide from spiked soil samples

Soil type	Recovery percentage				
	Fortification	Flubendiamide		Desiodo flubendiamide	
	Level (µg g^{-1})	Mean* ± S.D	RSD (%)	Mean* ± S.D	RSD (%)
New alluvial	0.01	87.47 ± 1.31	1.49	90.73 ± 1.57	1.73
	0.05	87.97 ± 1.26	1.43	87.51 ± 2.56	2.93
	0.10	88.58 ± 1.42	1.61	93.25 ± 1.18	1.27
Red and lateritic	0.01	89.05 ± 1.56	1.75	91.37 ± 0.98	1.08
	0.05	86.77 ± 1.82	2.09	88.76 ± 1.21	1.36
	0.10	93.30 ± 1.08	1.16	96.60 ± 2.01	2.08
Coastal saline	0.01	89.62 ± 0.68	0.76	93.08 ± 0.78	0.84
	0.05	89.65 ± 1.59	1.77	89.45 ± 1.23	1.37
	0.10	91.34 ± 1.23	1.35	94.34 ± 1.28	1.36

*Means of three replications, RSD- Relative standard deviation

desiodo flubendiamide in different soils viz. new alluvial,red and lateric and coasta saline soil and in water at different pH values, viz. 4, 7 and 9.2 by using HPLC. The accuracy and precision of the method was evaluated on the basis of the recoveries obtained for fortiûed soil and water samples. Soil samples were fortified with 0.01, 0.05 and 0.1 µg $g^1$of flubendiamide and desiodo flubendiamide. After evaporation of the spiking solvent, the samples were allowed to equilibrate for 1 h before extraction and analyzed following the procedures described above. Fortiûed samples were processed and analyzed in triplicate. The recoveries obtained for all pesticides ranged from 87.47 to 93.25% for new alluvial soil, 86.77 to 96.6% for red and lateritic soil, and 89 to 94% for coastal soil (Table 1). The mean relative standard deviations (RSD) did not exceed 2.09 % and 2.93 % for flubendiamide and desiodo flubendiamide, respectively.

In water fortified with 0.01-0.1 µg mL^{-1}, recoveries were between 86 to 89% and 90 to 95 % for low spike levels and between 90 to 93 % and 94 to 97% for high spike levels of flubendiamide and desiodo flubendiamide respectively (Table 2). The mean relative standard deviations did not exceed 2.80 % and 2.01 % for flubendiamide and desiodo flubendiamide, respectively.

A rapid and sensitive isocratic reversed-phase HPLC method has been developed herein for the determination of flubendiamide and desiodo flubendiamide in soil and water at different pH. Good recovery and low LOQs were obtained for both the pesticides studied. Results from validation showed recovery was excellent (86.77-96.60%) in soil and for water 86.02-97.25%, and precision was good (RSD < 3.0%), meeting directives for method validation in pesticide residue analysis. The proposed analytical procedure could be utilized for monitoring of flubendiamide and its metabolites in soil and water samples.

Table 2. Recoveries and RSD of flubendiamide from spiked water samples

pH	Recovery percentage				
	Fortification	Flubendiamide		Desiodo flubendiamide	
	Level (µg g^{-1})	Mean* ± S.D	RSD (%)	Mean* ± S.D	RSD (%)
4.0	0.01	86.02 ± 1.74	2.02	90.08 ± 1.81	2.01
	0.05	89.70 ± 0.88	0.99	91.78 ± 1.11	1.21
	0.10	91.92 ± 1.46	1.59	94.90 ± 1.48	1.56
7.0	0.01	88.22 ± 2.47	2.80	93.77 ± 1.40	1.50
	0.05	88.95 ± 0.82	0.92	93.46 ± 0.81	0.86
	0.10	90.66 ± 1.14	1.26	94.31 ± 1.24	1.32
9.2	0.01	89.16 ± 1.33	1.49	93.62 ± 1.32	1.41
	0.05	92.13 ± 1.25	1.36	95.84 ± 1.46	1.53
	0.10	95.66 ± 1.21	1.26	97.25 ± 1.25	1.28

*Means of three replications, RSD- Relative standard deviation

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