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Simple and Efficient Method for the Estimation of Residues of Flubendiamide and Its Metabolite Desiodo Flubendiamide

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An analytical method was standardized for the estimation of residues of flubendiamide and its metabolite desiodo flubendiamide in various substrates comprising cabbage, tomato, pigeonpea grain, pigeonpea straw, pigeonpea shell, chilli, and soil. The samples were extracted with acetonitrile, diluted with brine solution, and partitioned into chloroform, dried over anhydrous sodium sulfate, and treated with 500 mg of activated charcoal powder. Final clear extracts were concentrated under vacuum and reconstituted into HPLC grade acetonitrile, and residues were estimated using HPLC equipped with a UV detector at 230 λ and a C_{18} column. Acetonitrile/water (60:40 v/v) at 1 mL/min was used as mobile phase. Both flubendiamide and desiodo flubendiamide presented distinct peaks at retention times of 11.07 and 7.99 min, respectively. Consistent recoveries ranging from 85 to 99% for both compounds were observed when samples were spiked at 0.10 and 0.20 mg/kg levels. The limit of quantification of the method was worked out to be 0.01 mg/kg.

KEYWORDS: Acetonitrile; chloroform; desiodo flubendiamide; flubendiamide; HPLC; HPTLC

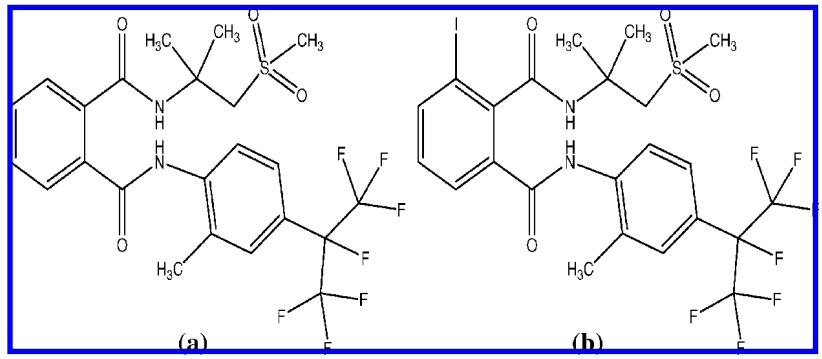

Figure 1. Chemical structures of (**a**) desiodo flubendiamide and (**b**) flubendiamide

INTRODUCTION

Flubendiamide, *N*′-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-*N*-{4-[2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-0-tolyl}phthalimide belongs to a new chemical class, the phthalic acid diamides, and is widely used against lepidopteran pests on a variety of annual and perennial crops (**Figure 1**). It provides superior plant protection against a broad range of economically important lepidopterous pests including *Helicoverpa* spp., *Heliothis* spp., *Spodoptera* spp., *Plutella* spp., *Trichoplusia* spp., and *Hyrotis* spp. Experiments in North America have shown flubendiamide to be hydrolytically stable, relatively immobile in soil, and practically nondectable in key rotated crops. Flubendiamide has a favorable ecological, ecotoxicological, and environmental profile with low mammalian toxicity and no genotoxic, mutagenic, or oncogenic properties noted (*1*). With its widespread use in mind, it was planned to standardize the methodology for the estimation of residues of flubendiamide and its metabolite desiodo flubendiamide on cabbage, tomato, pigeonpea, chilli, and soil.

EXPERIMENTAL PROCEDURES

Reagents and Materials. Acetonitrile was obtained from E. Merck Chemicals. Acetonitrile of HPLC grade was also from E. Merck Chemicals. Chloroform was obtained from SD Fine Chemicals. Activated charcoal powder was from Qualigens. Sodium sulfate

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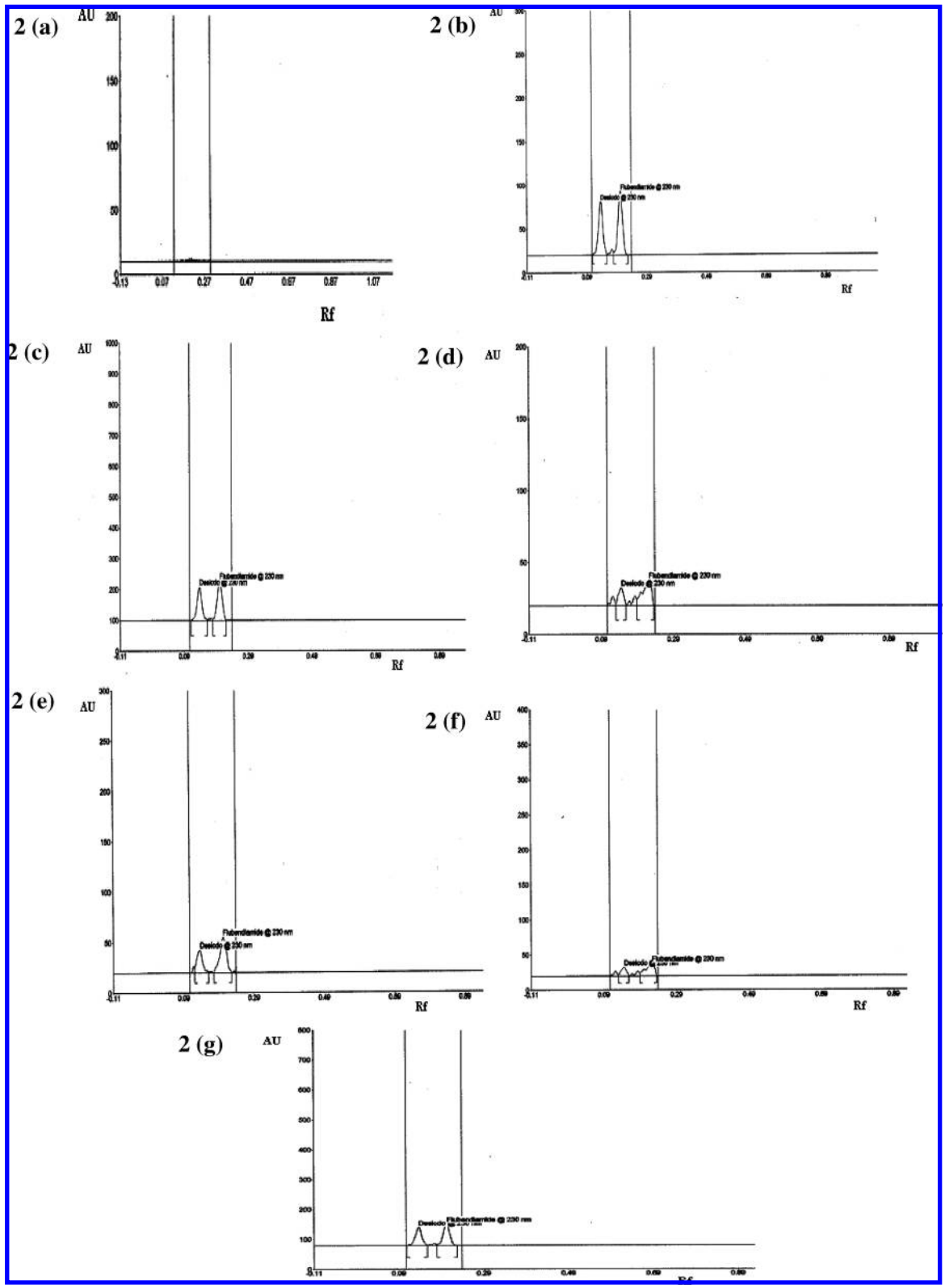

Figure 2. HPTLC chromatograms of (**a**) untreated and (**b**) standard desiodo flubendiamide and flubendiamide 100 ng each, (**c**) soil, (**d**) chilli, (**e**) tomato, (**f**) pigeonpea, and (**g**) cabbage, each spiked with desiodo flubendiamide and flubendiamide.

(anhydrous) was also from E. Merck Chemicals. The HPLC column, a Luna 5 μm $C_{18(2)}$ column, 250 × 4.6 mm (carbon load 17.5%), was obtained from Spincotech Pvt. Ltd. Chennai (India). Water of HPLC grade was from E. Merck Chemicals.

Instrumentation. The high-performance liquid chromatograph (HPLC) was from Shimadzu and was equipped with a RP $C_{18(2)}$ column and a UV detector photodiode array (PDA), dual pump. The high-performance thin layer chromatograph (HPTLC) was from CAMAG. The rotary vaccum film evaporator was a Perfit model GSIBU-6D.

Standard Compounds. Flubendiamide and desiodo flubendiamide having analytical purities of 99.8 and 99.2%, respectively, were obtained from M/s Bayer Crop Science (India) Limited, Mumbai.

Residue Analysis. Cabbage, tomato, pigeonpea grains, shell, and straw, chilli, and soil samples were used as substrates for standardization of the methodology for estimation of flubendiamide and its metabolite desiodo flubendiamide. The detailed procedure is described below.

Table 1. Recoveries of Flubendiamide and Desiodo Flubendiamide from Cabbage, Tomato, Pigeonpea Grain, Straw, and Shell, Chilli, and Soil

substrate	level of fortification, mg/kg	recovery %[a] flubendiamide	recovery %[a] desiodo flubendiamide
cabbage	0.1	95.27 ± 2.40	97.20 ± 4.68
	0.2	96.50 ± 3.10	98.44 ± 3.50
tomato	0.1	85.00 ± 4.58	88.84 ± 4.26
	0.2	92.40 ± 2.80	94.40 ± 3.15
pigeonpea grain	0.1	96.13 ± 2.50	97.33 ± 3.18
	0.2	99.33 ± 1.15	98.48 ± 2.48
pigeonpea straw	0.1	86.53 ± 4.80	87.82 ± 3.72
	0.2	85.92 ± 3.20	86.66 ± 2.78
pigeonpea shell	0.1	91.77 ± 1.84	90.80 ± 2.20
	0.2	93.37 ± 2.66	94.36 ± 3.60
chilli	0.1	94.36 ± 2.12	96.80 ± 3.16
	0.2	92.82 ± 2.72	95.72 ± 3.82
soil	0.1	94.67 ± 3.05	90.45 ± 4.78
	0.2	98.37 ± 2.66	96.80 ± 3.16

[a] Each value is the mean ± standard deviation of six replicate determinations.

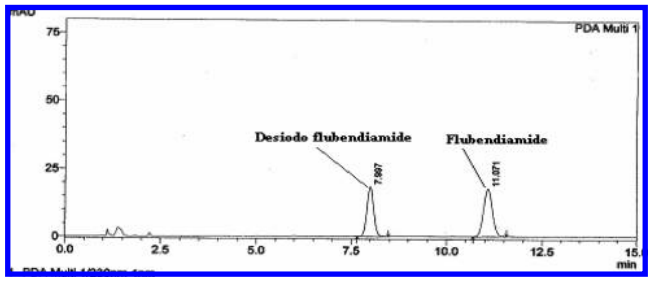

Figure 3. Chromatograms of standard of desiodo flubendiamide and flubendiamide, 2 ng each.

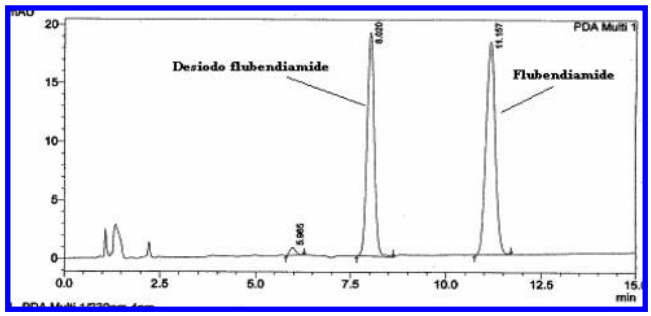

Figure 4. Chromatograms of standard of desiodo flubendiamide and flubendiamide, 20 ng each.

Extraction and Cleanup. A representative 50 g sample of chopped and macerated cabbage/tomato/pigeonpea grain, shell, straw/chilli/soil was placed into 100 mL of acetonitrile in an Erlenmeyer flask for 24 h. The extract was filtered into a 1 L separatory funnel along with rinsings of acetonitrile. The filtrate in the separatory funnel was diluted with 600 mL of brine solution (almost saturated sodium chloride solution), and the contents were partitioned three times into 100, 50, and 50 mL of chloroform. The chloroform fractions were combined, dried over anhydrous sodium sulfate, and treated with 500 mg of activated charcoal powder for about 2–3 h at room temperature. The clear extract so obtained was filtered through Whatman filter paper no.1 and concentrated to near dryness, and the residues were dissolved in 20 mL of HPLC grade acetonitrile and again concentrated using the rotary vacuum

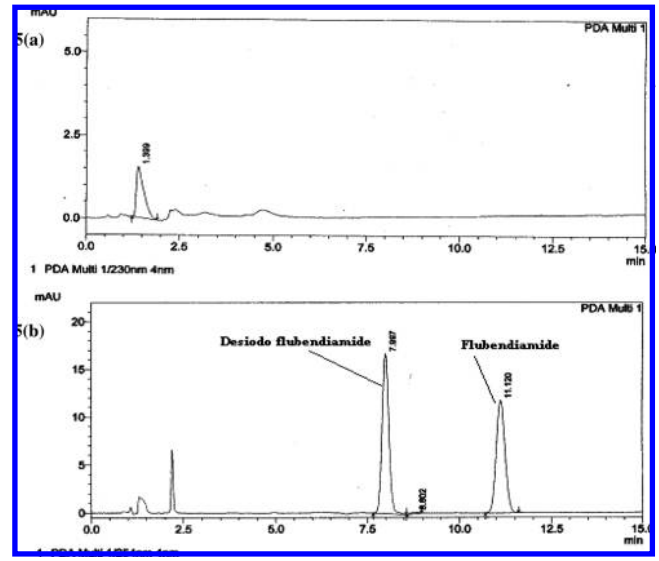

Figure 5. Chromatograms of (**a**) untreated and (**b**) soil samples spiked with desiodo flubendiamide and flubendiamide.

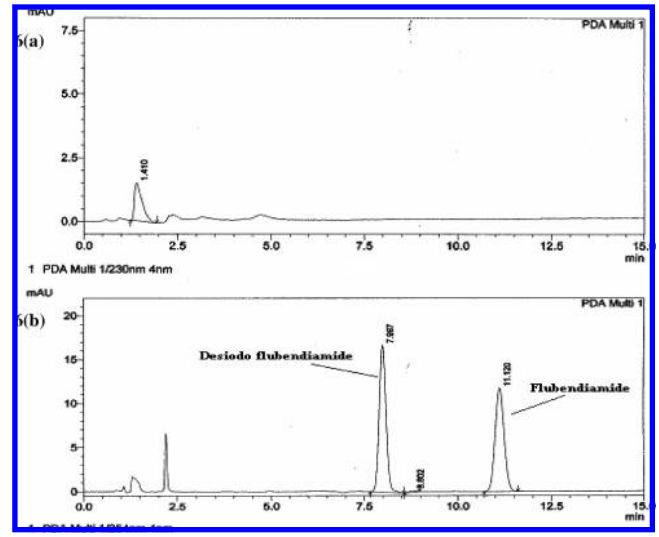

Figure 6. Chromatograms of (**a**) untreated and (**b**) tomato samples spiked with desiodo flubendiamide and flubendiamide.

evaporator at 30 °C. The process was repeated to completely evaporate chloroform, and the final volume was reconstituted to about 5−10 mL using HPLC grade acetonitrile.

Estimation of Residues. The residues of flubendiamide and its metabolite desiodo flubendiamide were estimated on HPLC by employing a Phenomenex Luna C_{18} column, a UV detector at 230 λ (wavelength), and an acetonitrile/water (60:40, v/v) mixture as mobile phase at 1 mL/min. Under these operating conditions the retention times of flubendiamide and desiodo flubendiamide were found to be 11.07 and 7.99 min, respectively. Residues were estimated by comparison of peak height/peak area of the standards with that of the unknown or spiked samples run under identical conditions. Half-scale deflection was obtained for 5 ng of flubendiamide, and the limit of quantification (LOQ) was found to be 0.01 mg/kg. Percent recoveries and standard deviations of flubendiamide and desiodo flubendiamide from cabbage, tomato, pigeonpea grain, straw, and shell, chilli, and soil are presented in **Table 1**.

Confirmation. The residues of flubendiamide and desiodo flubendiamide were confirmed by HPTLC. This technique was able to identify and quantify 100 ng each of flubendiamide and desiodo flubendiamide. Cleaned up sample extracts of different substrates were spotted on precoated silica gel on aluminum sheets along with reference standards of flubendiamide and desiodo flubendiamide, visualized through a scanner (TLC Scanner 3, D_2 lamp with wavelength range of 190–400

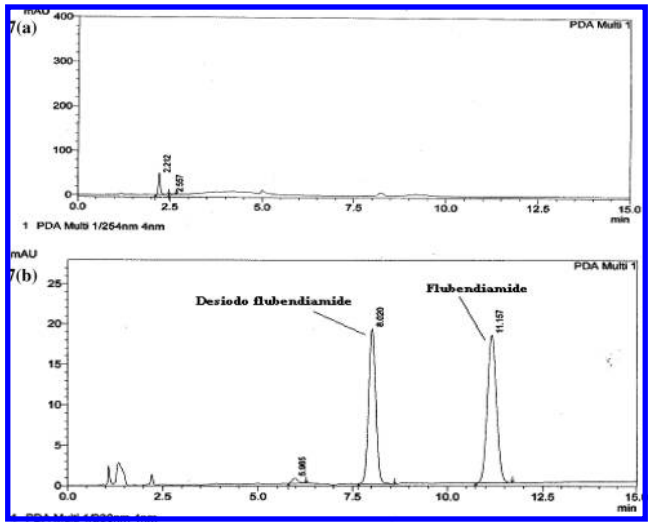

Figure 7. Chromatograms of (**a**) untreated and (**b**) pigeonpea samples spiked with desiodo flubendiamide and flubendiamide.

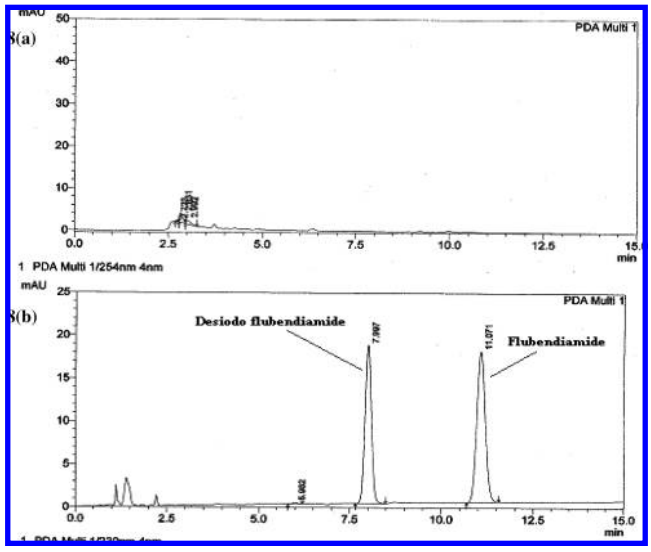

Figure 8. Chromatograms of (**a**) untreated and (**b**) chilli samples spiked with desiodo flubendiamide and flubendiamide.

nm), and quantified by comparison of the peak height/area of the sample with those of reference standards under similar conditions (**Figure 2**).

RESULTS AND DISCUSSION

Flubendiamide is a new lepidopteran insecticide that is being developed for use in a broad number of annual and perennial crops and belongs to a new chemical class, the phthalic acid diamides (*1*). Residues of flubendiamide and its metabolite desiodo flubendiamide were determined in a number of crops on which its use is expected to be high in the future. The crops thus selected included cabbage, tomato, pigeonpea, chilli, and soil as these crops are affected by lepidopteran pests.

Initially, the representative samples of these crops were placed in acetone and partitioned in dichloromethane, but the recoveries

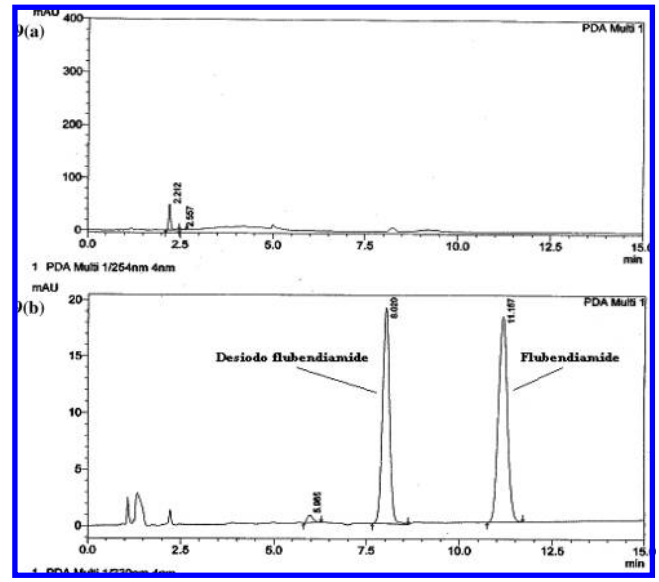

Figure 9. Chromatograms of (**a**) untreated and (**b**) cabbage samples spiked with desiodo flubendiamide and flubendiamide.

were observed to be very poor, that is, to the extent of only 20%. Later on, ethyl acetate and hexane were also tried, but the percent recoveries were again very poor. The chemical structure of flubendiamide (**Figure 1**) and its synthetic route were studied (*2*), and consequently a representative 50 g sample of tomato was spiked with flubendiamide and desiodo flubendiamide at 0.1 and 0.2 mg/kg level,, extracted with acetonitrile, and partitioned into chloroform (100, 50, and 50 mL). The results were astonishing as the percent recoveries ranged from 85 to 94. The same method was applied to other substrates, and the results were found to be excellent. The percent recoveries of flubendiamide and desiodo flubendiamide from different substrates spiked at 0.1 and 0.2 mg/kg are reported in **Table 1**, and each value is the mean ± standard deviation of six replicate determinations (**Figures 3**−**9**). The results were encouraging and suggested that the method could be extended to more substrates. Moreover, this seems to be the first report regarding the estimation of residues of flubendiamide and its metabolite desiodo flubendiamide according to a method that is simple, efficient, and easy to adopt in laboratories engaged in pesticide residue analysis.

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