IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 5 Ver. II (May. 2015), PP 49-54
www.iosrjournals.org

A New Method for Determination of Flubendiamide and Its Metabolite Residues in Jatropha Plant Leaves

Tentu. Nageswara Rao[1]*, Karri Apparao[1] and SNVS. Murthy[2]
[1]*Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India.*
[2] *Department of Chemistry, DLR P.G College, Gollalamamidada, Andhra Pradesh, India.*

Abstract: *A simple and inexpensive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with UV detection for determination of flubendiamide and its metabolite (flubendiamide- des- iodo) residues. The evaluated parameters include the extracts by alumina packed column using hexane: ethyl acetate solvent mixture (9:1) and acetonitrile solvent. The method was validated using leaf samples spiked with flubendiamide and its metabolite (flubendiamide- des- iodo) at different fortification levels (0.03 and 0.3 µg/g). Average recoveries (using each concentration six replicates) ranged 85-96%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.03-10.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01µg/g and 0.03µg/g respectively. Finally the leaf residue samples were re analyzed by HPLC.*

Key words*: HPLC, Alumina, Flubendiamide and its metabolite and jatropha plant leaves.*

I. Introduction

Jatropha curcas also known as raranjot, van errand or moghal errand, it is multipurpose plant with many attributes and considerable potential. It is a tropical plant that can be grown in low to high rainfall areas and can be used to reclaim land, as a hedge and commercial crop. Thus, growing it could provide employment, improve the environment and enhance the quality of rural life. The establishment, management and productivity of jatropha under various climatic conditions are not fully documented. This is discussed and the gaps in the knowledge elucidated, especially its fertilizer requirements [1]. The plant produces many useful products, especially the seed, from wich oil can be extracted. This oil has similar properties to palm oil. The oil extracted from the seed is non edible and is used in making soaps, cosmetics, colors, candles and wool. The oil is used in making plastics and synthetic fibers. Jatropha oil characteristics are rich in medicinal properties and are used in treatment of skin diseases, paralysis, toothache, stomachache etc [2].

Flubendiamide belongs to a chemical family of benzedicarboxamides or phthalic acid diamides with insecticidal activity through the activation of the ryanodine-sensitive intracellular calcium relese channels, leading to the cessation of feeding immediately after ingestion of the compound [3-5]. The compound was evaluated as a new compound by the 2010 JMPR for both residues and toxicological aspects [6]. Flubendiamide is a new insecticide that has been found to give excellent control of lepidopterous pests of Jatropha plant. This study has been undertaken to develop an improved methor for analysis of flubendiamide and its metabolite flubendiamide- des iodo and determine residueretention in Jatropha leaf.

II. Experimental

2.1 Standards, Reagents and samples

The analytical standards of flubendiamide (99.8%) and flubendiamide- des- iodo (99.2%) were obtained from Sigma Aldrich. Acetonitrile was purchased from Rankem, New Delhi, analytical grade solvents i.e., ethyl acetate, hexane and alumina sorbet were supplied from Merck Limited and jatropha plant leaves were collected from local jatropha plant field.

2.2 Standard stock solutions

The flubendiamide and flubendiamide- des- iodo standard stock solutions were individually prepared in acetonitrile at a concentration level 1000 µg/mL and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

2.3 Sample preparation

Representative 20.0 g portions of jatropha leaves fortified with 0.1 mL of working standard solutions. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

2.4 Extraction procedure

A 20g of representative Jatropha leaf sample was extracted with 100ml acetonitrile containing 0.002M hydrochloric acid twice by using an end-over-end mechanical shaker for 30 minutes. The extraction mixture was filtered and evaporated to near dryness using a vacuum rotary evaporator and the contents were re-dissolved in 20mL of acetonitrile.

2.5 Clean-up procedure

A glass column was packed with 10g of alumina using hexane as solvent. Drained the excess of solvent. The sample was transferred into the column and eluted with 50mL of 9:1 hexane: ethyl acetate solvent mixture. Discarded the first 10ml fraction and collected the elute over anhydrous sodium sulphate, this process was repeated thrice. Approximately 150ml of elute was collected. The elute was completely drained and evaporated to near dryness and then re-dissolved in 20 mL of acetonitrile. The sample was filtered through 0.45 µm filter and analyzed by HPLC.

2.6 Chromatographic separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC-20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (Phenomenex Luna-C18) Column temperature was maintained at 30°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and HPLC grade water (70:30 (v/v)). The flow- rate used was kept at 1.2 mL/min. A detector wavelength was 205 nm. The calibration curve method was used for determination of flubendiamide and its metabolite (flubendiamide- des- iodo) residues in leaf.

2.7 Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.03 and 0.3 mg/kg. Linearity was determined by different known concentrations (0.03, 0.1, 0.5, 1.0, 5.0 and 10.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise [7-8].

III. Results And Discussion

3.1 Specificity

Aliquots of flubendiamide and its metabolite (flubendiamide- des- iodo) standard solutions, spiking sample solution, leaf control, extracted solvents and mobile phase solvents were assayed to check the specificity. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in (**Figure 1 and 2).** Furthermore, the retention times of flubendiamide and flubendiamide- des- iodo were constant at 5.3 ± 0.2 and 4.3 ± 0.2, minutes.

3.2 Linearity

3 .2.1 Preparation of Flubendiamide standard stock solution

Accurately weighed 10.02 mg of reference analytical standard of flubendiamide in 10mL volumetric flask and dissolved in acetonitrile, sonicated and made upto the mark with the same solvent. The concentration of the stock solution was 1000 µg/mL.

3 .2.2 Preparation of Flubendiamide- des- Iodo Metabolite stock solution

Accurately weighed 10.08 mg of reference analytical standard of flubendiamide- des- Iodo in 10mL volumetric flask and dissolved in acetonitrile, sonicated and made upto the mark with the same solvent. The concentration of the stock solution was 1000 µg/mL.

3 .2.3 Preparation of Calibration solutions

Different known concentrations of standard solutions (0.03, 0.1, 0.5, 1.0, 5.0 and 10.0 µg/mL) were prepared in acetonitrile by diluting the above stock solutions. The serial dilution details were presented in **Table 1.**These standard solutions were directly injected into a HPLC. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions [9]. The peak areas obtained from different concentrations of standards were used to calculate linear regression equations. These were Y=8202.32X + 25.58 and Y=10362.83 + 27.52, with

correlation coefficients of 0.9999 and 1.0000 for flubendiamide and flubendiamide- des- Iodo respectively. A calibration curve showed in (**Figure 3).**

3.3 Accuracy and Precision

Recovery studies were carried out at 0.03 and 0.3 µg/mL fortification levels for flubendiamide and flubendiamide- des- Iodo in leaf. The recovery data and relative standard deviation values obtained by this method are summarized in **Table 1.**

These numbers were calculated from four (6) replicate analyses of given sample (flubendiamide and flubendiamide- des- Iodo) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<2 %).

3.4 Detection and Quantification Limits

The limit of quantification was determined to be 0.03 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (85-96%, RSD<2%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.03 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

3.5 Storage Stability

A storage stability study was conducted at refrigerator condition (5 ± 3°C) and Ambient temperature (25 ± 5°C) of 0.1 µg/g level fortified leaf samples were stored for a period of 30 days. Analysed for the contents of flubendiamide and flubendiamide- des- Iodo before storing and at the end of storage period [10]. The percentage dissipation observed for the above storage period was only less than 3% for flubendiamide and flubendiamide- des- Iodo showing no significant loss of residues on storage. The results are presented in T**able 3 and 4.**

IV. Calculations

The concentration of acetaminophen in the samples analyzed by HPLC was determined directly from the standard curve.

$Y = mx + c$

Where,

Y = peak area of standard (mAU*sec)

m = the slope of the line from the calibration curve

x = concentration of injected sample (mg/L)

c = 'y' intercept of the calibration curve

The recovered concentration or Dose concentration was calculated by using the formula:

$$\text{Recovered concentration or Dose concentration} = \frac{(x-c)\ X\ D\ X\ 100}{m\ X\ P}$$

Where,

m = the slope of the line from the calibration curve

x = sample area of injected sample (mAU*sec)

c = 'y' intercept of the calibration curve

D = Dilution Factor

P = Purity of Test item

$$\%\ \text{Recovery} = \frac{\text{Recovered Concentration}}{\text{Fortified Concentration}} \times 100$$

V. Conclusions

This paper describes a fast, simple sensitive analytical method based on SPE-HPLC-UV simultaneous determination of flubendiamide and flubendiamide- des- Iodo residues in jatropha plant leaves The SPE extraction procedure is very simple and inexpensive method for simultaneous determination of flubendiamide and flubendiamide- des- Iodo residues in jatropha plant leaves. The mobile phase Acetonitrile and HPLC grade water showed good separation and resolution and the analysis time required for the chromatographic determination of the flubendiamide and flubendiamide- des- Iodo is very short (around 15 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and very low

limits were obtained and according to the SANCO guidelines [14]. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of flubendiamide and flubendiamide- des- Iodo residues on a large number of leaf, seed, oil, fruit, water and soil samples [11-13].

Acknowledgement

The authors are thankful to the Dr. Gowtham Prasad, S.V.V University, Hyderabad for his keen interest and help.

References

[1]. Keith Openshaw, Areview of jatropha curcas: an oil plant of unfulfilled promise, Biomass and Bio energy, 19(1), 2000, 1-15.
[2]. D.M Reddy Prasad, Amirah Izam and Md. Maksudur Rahman Khan, Jatropha curcas: Plant of medical benfits, Journal of medicinal plants research, 6(14), 2012, 2691-2699.
[3]. Rubaljot Kooner, S.K.Sahoo, Balwinder Singh and R.S. Battu, Dissipation kinitics of flubendiamide and thiacloprid on tomato and soil, Quality assurance and safety of crops & foods, 2(1), 2010, 36-40.
[4]. S. Mohapatra, A.K. Ahuja, G.K. Jagadish, N. Rashmi, D. Sharma, Development of an analytical method for analysis of flubeniamide, des-iodo flubendiamide and study their residue persistence in tomato and soil, J Environ Sci Health B, 46(3), 2011, 264-271.
[5]. Paramasivam, M, Benerjee, Hemantha, Simultaneous determination of flubendiamide its metabolite desiodo flubendiamide residues in cabbage, Tomato and pigeon pea by HPLC, Bulletin of environmental contamination &Toxicology, 87 (4), 2011, 452.
[6]. Xiaojun Chen, et al, Degradation dynamics and residue analysis of flubendiamide in cabbage and soil by liquid chromatography-tandem mass spectrometry with dispersive solid phase extraction, Agricultural Sciences, 5, 2014, 850-857.
[7]. Tentu Nageswara Rao, et al, Persistence study of pyraclostrobin and epoxiconazole fungicide formulation in groundnut plant followed by HPLC-UV method, International journal of current microbiology and applied sciences, 2(9), 2013, 5-13.
[8]. Tentu. Nageswara Rao, T. Srinivasa Rao and G. Silpa, Determination of lymecycline and tetracycline residues in bovine milk followed by matrix solid-phase dispersion coupled to highperformance liquid chromatography with ultraviolet detection. World journal of pharmaceutical research 1(5), 2012, 1281-1290.
[9]. Sannino A, et al, Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of a new generation of pesticides in processed fruits and vegetables, Journal of Chromatography A, 1036, 2004,161-169.
[10]. Tentu. Nageswara Rao, D. Sreenivasulu, T.B. Patrudu and E.G. Sreenivasula Reddy Simultaneous Extraction and detection of six fungicide residues in mango fruit Followed by new Validated HPLC-UV method, Scholars Academic Journal of Bioscience 1(3), 2013, 80-84.
[11]. Muccio AD, et al, Application of solid-phase extraction and liquid chromatography–mass spectrometry to the determination of neonicotinoid pesticide residues in fruit and vegetables, Journal of Chromatography A, 1108, 2006, 1-6.
[12]. Jian Pan, et al, Analysis of pesticide multi-residues in leafy vegetables by ultrasonic solvent extraction and liquid chromatography-tandem mass spectrometry. Ultrasonics Sonochemistry, 15, 2008, 25-32
[13]. Fernandez, M et al., Comparison of gas and liquid chromatography coupled to mass spectrometry for the residue analysis of pesticides in oranges. Chromatographia 54(5), 2001, 302-308.
[14]. SANCO Guidelines, Method validation and quality control procedures for pesticide residues analysis in food and feed. Document NO. SANCO/10684/2009.

Fig.1. Representative Chromatogram of jatropha leaf control

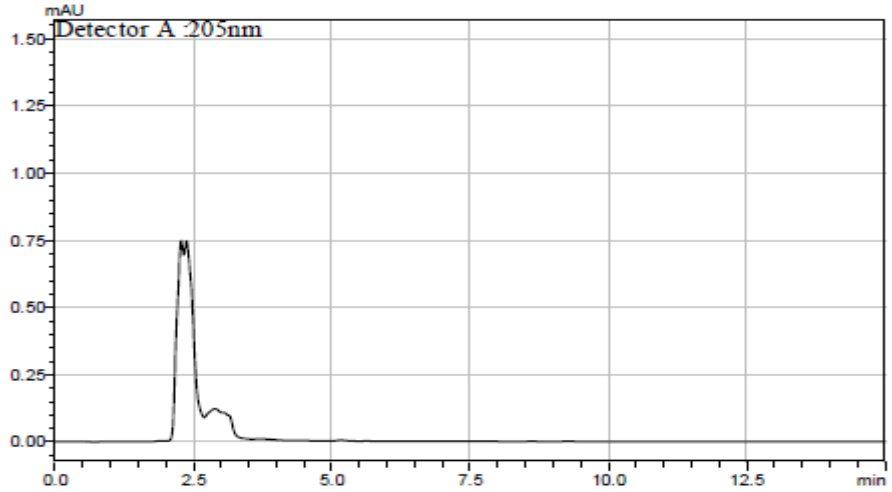

Fig.2. Representative Chromatogram at fortification level of 0.03 µg/g

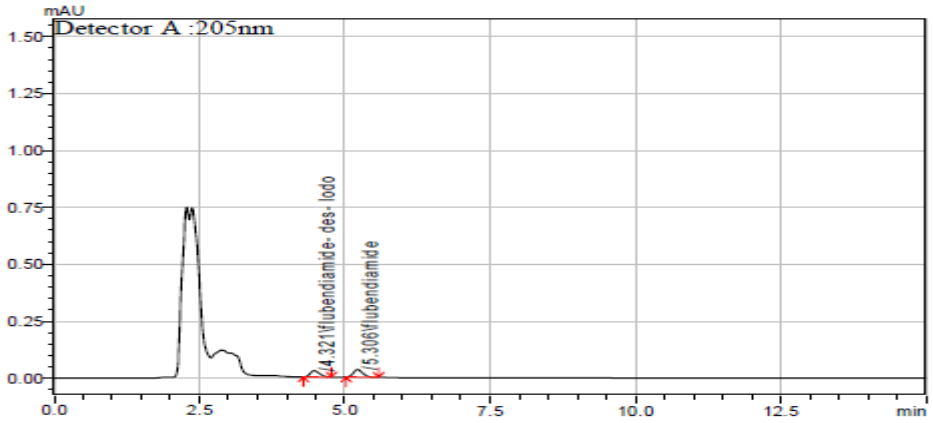

Fig.3. Representative Calibration curve of flubendiamide and flubendiamide- des- Iodo

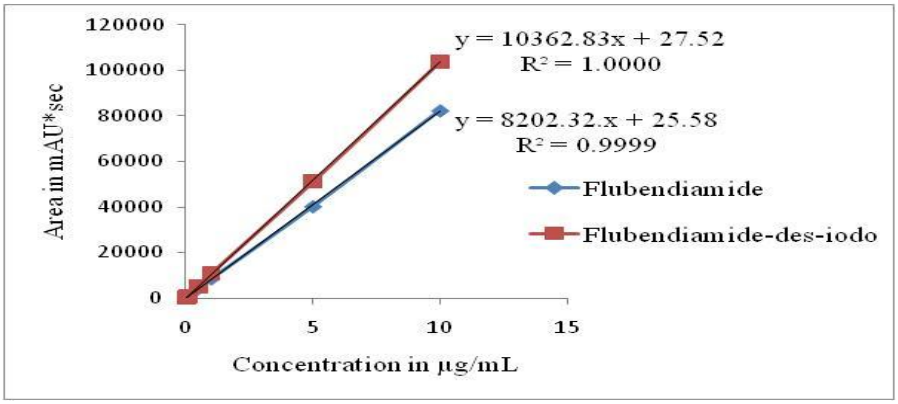

Table 1. Serial dilutions of linearity standard solutions

Stock solution concentration (µg/mL)	**Volume taken from stock solution (mL)**	**Final make up volume (mL)**	**Obtained concentration (µg/mL)**
1000	1.000	10	100
100	1.000	10	10
100	0.500	10	5
100	0.100	10	1
10	0.5	10	0.5
10	0.1	10	0.1
1	0.3	10	0.03

Table 2. Recoveries of the flubendiamide and flubendiamide- des- iodo from fortified Jatropha leaf control sample (n=6)

Fortification Concentration in µg/mL	Replication	Recovery (%)	
		flubendiamide	flubendiamide- des- iodo
	R1	85	86
	R2	84	86
	R3	86	87
0.03	R4	88	85
	R5	84	84
	R6	85	86
	Mean	**85.33**	**85.67**
	RSD	**1.76**	**1.21**
	R1	95	96
	R2	94	98
	R3	96	97
0.3	R4	97	96
	R5	97	94
	R6	96	95
	Mean	**95.83**	**96.00**
	RSD	**1.22**	**1.47**

Table3. Storage stability Details at refrigerator condition (5 ± 3°C)

Fortification Concentration in µg/mL	Storage Period in Days	Recovery in %	
		flubendiamide	flubendiamide- des- iodo
		95	95
		94	94
		95	92
		93	92
	0	96	96
		98	97
	Average	**95.2**	**94.3**
	STDEV	**1.72**	**2.07**
	RSD in %	**1.81**	**2.19**
0.1		92	90
		93	91
		92	90
	30	91	92
		91	91
		90	91
	Average	**91.5**	**90.8**
	STDEV	**1.05**	**0.75**
	RSD in %	**1.15**	**0.83**

Table 4. Storage stability Details at ambient Temperature (25 ± 2°C)

Fortification Concentration in µg/mL	Storage Period in Days	Recovery in %	
		flubendiamide	flubendiamide- des- iodo
		94	94
		93	93
		94	93
		95	94
	0	95	95
		96	95
	Average	**94.5**	**94.0**
	STDEV	**1.05**	**0.89**
	RSD in %	**1.11**	**0.95**
0.1		91	89
		92	92
		91	91
	30	92	91
		92	90
		90	90
	Average	**91.3**	**90.5**
	STDEV	**0.82**	**1.05**
	RSD in %	**0.89**	**1.16**