Research Article
ISSN (Print): 0971-6580
Toxicology International, *Vol 27(1&2), 23-27, 2020, DOI: 10.18311/ti/2020/v27i1&2/24930* ISSN (Online): 0976-5131

Development and Validation of Simple HPLC Method for Estimation of Flubendiamide in Blood

R. Mandil[1*], A. Prakash[2], R. K. S. Yadav[2], A. Rahal[3] and S. K. Garg[2]

[1]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Science, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut – 250110, Uttar Pradesh, India; rajesh_mandil@rediffmail.com

[2]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U. P. Pt. Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya, Evam Go-Anusandhan Sansthan (DUVASU), Mathura – 281001, Uttar Pradesh, India

[3]Central Institute for Research on Goat (CIRG), Makhdoom, Farah, Mathura – 281122, Uttar Pradesh, India

Abstract

Flubendiamide, novel insecticide used for control of lepidopteron insects on agriculture crops in India. Flubendiamide exposure of to human and animals occurs through ingestion of contaminated feed/fodder, food-products and drinking water etc. Aim of the present study was to develop a simple HPLC method for flubendiamide estimation in blood plasma. Result indicated that HPLC estimation of flubendiamide in blood plasma shows linear standard curve in the range of 0.039-5.0 $\mu g.ml^{-1}$ with correlation coefficient (R^2) 0.999. Limit of Detection (LOD) and Limit of Quantification (LOQ) was 0.039 μgml^{-1}. Intra-day, inter-day coefficient of variance and mean recovery were found to be 2.70%, 3.50% and approx. 100% respectively, by using C_{18} column and mixture of acetonitrile: water (70:30, v/v) as mobile phase with flow rate of 0.5 $mlmin^{-1}$ at 210 λ. Result of this study concluded that this HPLC method is very simple, reliable and repeatable for flubendiamide estimation blood plasma.

Keywords: Flubendiamide, Plasma, HPLC

1. Introduction

Flubendiamide (NNI 0001) N2-[1,1- dimethyl-2-(methyl-sulfonyl) ethyl]-3-iodo-N1-[2- ethyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl] phenyl]-1,2-benzene-dicarboxamide) recently attract good attention towards control of insects on more than 200 crops including rice, cotton, corn, grapes/fruits and vegetable crops, in India[1,2]. It is a fast and long last acting insecticide and has selective sensitivity towards ryanodine receptor of lepidopteron insect without affecting mammalian ryanodine receptors[3]. Among cereals grown in India, rice is one of most consumed crop and approximately 50% loss of rice crop is due to lepidopteron pests[4]. It's stable both under aerobic-anaerobic soil conditions and aerobic-aquatic laboratory conditions and very slowly degraded under field condition to des-iodo form[5]. Des-iodo form is ten times more toxic to aquatic invertebrates than flubendiamide[6]. Environmental persistence behaviour of flubendiamide and the residues level in soil and water act as a source of contamination of food chain[7]. Thus, contaminated rice-grains, agriculture crops and their products and fruits etc. are potential source of exposure of flubendiamide

*Author for correspondence

to human and animals[8–11]. Therefore, there is need to assess the impact of exposure effect of flubendiamide on different vital organs of human and animals through estimation of flubendiamide in blood.

Bayer Laboratories recommended the use of Liquid Chromatography/Mass Spectroscopy (LC/MS) method for detection of flubendiamide and its metabolite des-iodo flubendiamide in soil[12]. Several methods available for analysis of flubendiamide and its metabolites des-iodo flubendiamide in soil and vegetable crops[8,13,14], but no information on a HPLC method for the determination of flubendiamide in blood is available. Therefore, presents study was undertaken for development of simple High Performance Liquid Chromatography (HPLC) method for flubendiamide estimation in blood plasma.

2. Materials and Methods

2.1 Experimental Animals, Sample Collection and Extraction of Plasma Samples

Male Wistar rats were procured from Laboratory Animal Resource Section, Indian Veterinary Research Institute (IVRI), Izatnagar and maintained under standard managemental conditions in the Departmental Laboratory Animal House. Animals had free access to pelleted feed (Ashirwad Industries, Chandigarh) and clean and deionized drinking water. Daily light and dark cycle of 12h was ensured. Before start of the experiment, an acclimatization period of 15 days was allowed. The research work was conducted after approval from Institutional Animal Ethics Committee (Approval No. 79 IAEC/13), DUVASU, Mathura. Blood from experimental rats was collected in heparinised tubes from inner canthus of eye through retro-orbital plexus puncture with the help of glass capillary tubes after overnight fasting as per the method of Sorg and Buckner[15]. Plasma and buffy-coat were removed carefully from anticoagulant mixed blood samples after centrifugation at 2000 rpm for 10 min. Plasma was separated, transferred into 1.0 ml appendorf tubes and stored at -20^{0}C for further HPLC analysis of flubendiamide.

2.2 HPLC Analysis of Flubendiamide

2.2.1 Reagents, Solvents and Standards

HPLC grade of hexane, acetone, actonitrile were purchased from Merck-Millipore, India. Flubendiamide standard of HPLC grade was procured from the Sigma-Aldrich, India. All other chemicals used in this study were of HPLC grade.

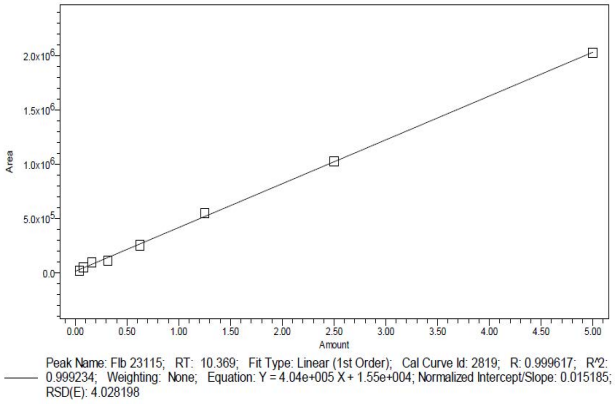
2.2.2 *Extraction, Clean Up and Chromatographic Conditions*

0.25 ml of plasma sample was deproteinized with 0.75 ml hexane:acetone (8:2) mixture. This mixture was vortexed for 1 min at high speed and centrifuged at 12000 rpm for 20 min. Mixture was separated into three distinct layers. The top layer contains clear supernatant was collected and filtered through Millipore 0.22 µm cellulose acetate membrane filter and an aliquot of 20 µl of sample was injected into Rheodyne manual loop injector of HPLC system. Chromatographic conditions in HPLC system (Waters, USA) consist of two Waters 515 HPLC pump with Waters 2996 Photodiode Array Detector (PDA) by employing C_{18} reverse phase column (particle size 5µm; 4.6 x 100 mm, Waters XBridge^{TM}) as a stationary phase at 210 λ (wavelength) and using acetonitrile: water (70:30, v/v) mixture as mobile phase with flow rate 0.5 mlmin^{-1}, and the samples were run for 13.0 minutes at column temperature (35±1.0°C).

2.2.3 *Preparation of Calibration Curve, Method Validation and Recovery*

Flubendiamide standard stock solution was prepared in acetonitrile and the final concentration of the stock solution was 10 µgml^{-1}. 0.5 ml plasma was taken from the stock flubendiamide standard (10µg/ml) solution and working plasma standards concentrations of flubendiamide in the range of 5.0 µgml^{-1}, 2.5 µgml^{-1}, 1.25 µgml^{-1}, 0.625 µgml^{-1}, 0.312 µgml^{-1}, 0.16 µgml^{-1}, 0.079 µgml^{-1} and 0.039 µgml^{-1} were prepared by serial dilution from the stock solution of flubendiamide with pooled plasma collected from untreated rats and similar extraction, cleanup and sample preparation procedure was followed. The calibration curve of flubendiamide was plotted using liner regression

in the range of 0.039 to 5.0 µg/ml of plasma. The standard curve was plotted using peak area (y-axis) against concentration of flubendiamide (x-axis) which was used for determination of flubendiamide concentration in unknown plasma samples (Figures 1 a, b). The software used for assay of flubendiamide was Empower.



(a)

Peak: Flb 23115

	Sample Name	Result Id	Peak Name	Level	X Value	Response	Calc. Value	% Deviation
1	0.03975 ug P 1 H8:A2 22115	2827	Flb 23115		0.040	22434.001	0.017	-56.51
2	0.07955 ug P 1 H8:A2 22115	2826	Flb 23115		0.080	51330.000	0.089	11.75
3	0.1575 ug P 1 H8:A2 22115	2825	Flb 23115		0.157	95408.001	0.198	26.11
4	0.3125 ug P 1 H8:A2 22115	2824	Flb 23115		0.313	112744.003	0.241	-22.91
5	0.625 ug P 1 H8:A2 22115	2823	Flb 23115		0.625	254655.997	0.592	-5.23
6	1.25 ug P 1 H8:A2 22115	2822	Flb 23115		1.250	554117.002	1.334	6.71
7	2.5 ug P 1 H8:A2 22115	2821	Flb 23115		2.500	1030056.995	2.512	0.50
8	5 ug P 2 H8:A2 22115	2820	Flb 23115		5.000	2026627.015	4.980	-0.40

(b)

Figure 1. **(a)** Calibration curve of flubendiamide in plasma of rats **(b)** Flubendiamide concentration µgml^{-1} in rat plasma for preparation of caliberation curve.

Method validation required accuracy, precision, linearity and LOD and LOQ. Accuracy of the present method was determined by recovery of flubendiamide, using samples spiked at concentration levels of 0.039 and 5.0 µgml^{-1}. Precision was determined by intraday and inter-day variation of analytical method by using two known concentrations (0.625 µg/ml and 2.5 µg/ml) at three different time intervals 24 hrs apart between any two assays. Linearity was determined by different known concentrations (5.0 µgml^{-1}, 2.5 µgml^{-1}, 1.25 µgml^{-1}, 0.625 µgml^{-1}, 0.312 µgml^{-1}, 0.16 µgml^{-1}, 0.079 µgml^{-1} and 0.039 µgml^{-1}) were prepared by diluting the stock solution. Limit of detection (LOD, µgml^{-1}) and limit of quantification (LOQ, µgml^{-1}) were determined as the lowest concentration to be detected and quantify by giving a response of 1:3 and 1:10 ratio of the base line noise from untreated sample[16].

Analytical recovery with this method was determined by adding flubendiamide to a solution of Hexane: acetone (8:2) mixture to yield concentration in the range of 0.04 to 5.0 µgml^{-1} and were processed like plasma samples. Both plasma and hexane-acetone (8:2) mixture added samples were analyzed simultaneously. For each flubendiamide concentration, three replicates were made. Recovery was estimated as ratio of peak areas obtained from plasma and those obtained from hexane-acetone mixture and percent recovery was calculated by the formula-

$$\% \text{ Recovery } = \frac{\left\{\sum x.y - \left(\sum x.\frac{\sum y}{n}\right)\right\}}{\left\{\sum x^2 - \frac{(\sum x)^2}{n}\right\}}$$

where,

x = concentration in hexane-acetone mixture

y = concentration in plasma samples

n = number of sample sets

3. Results and Discussion

The flubendiamide was detected at 10.36 min during sample run in HPLC system for 13.0 min. Blank plasma, representative chromatogram and overlay peaks of flubendiamide standard in plasma is depicted in Figures 2, 3 and 4, respectively. No interfering peaks from endogenous substance were observed at the retention time. The correlation coefficient (R) was 0.999 and the intra-day and inter-day coefficient of variance were found to be 2.70% and 3.50% respectively, and the mean recovery was approximately 100.00%±13.05. LOD and LOQ were 0.039 µgml^{-1}.

Perusal of result indicated that retention time of flubendiamide was 10.36 min and LOD and LOQ were almost similar i.e. 0.039 µgml^{-1} which was very less

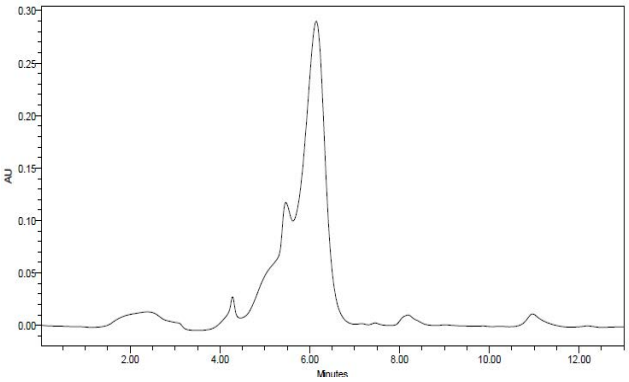


Figure 2. HPLC chromatogram showing blank plasma.

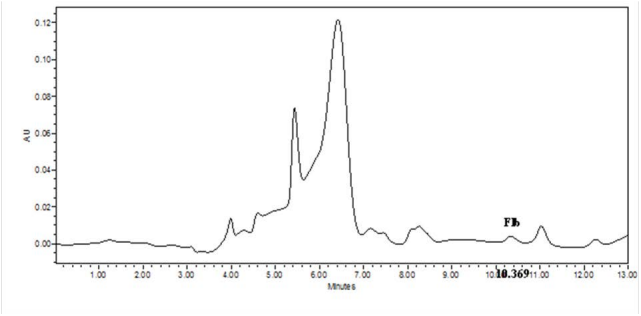


Figure 3. Representative chromatogram showing fluben diamide peak at 0.079 μgml^{-1}.

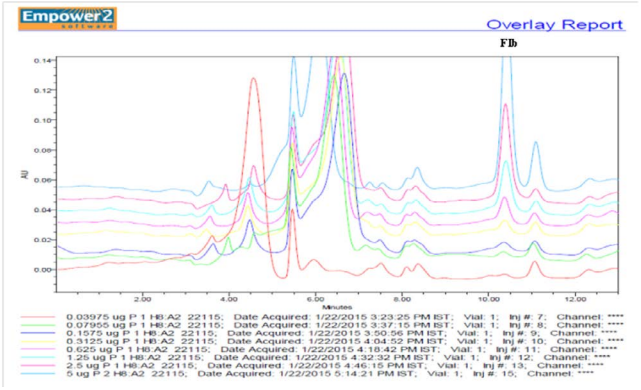


Figure 4. Chromatogram showing overlay of peaks and retention time of flubendiamide (Flb) standards in plasma of rats.

compared to limit of quantification by Motoba[17] i.e. 0.1 mg/l or mg/kg at the dose rate of 200 mg/kg in plasma and liver of rats and mice and in fat. Residues in plasma samples can be estimated by comparison of peak height/ peak area of the standards with that of the unknown samples run under identical conditions. Thus, present method of flubendiamide estimation is very simple, efficient, extraction less and repeatable and useful for detection and impact assessment on different tissues/ organs of humans and animals following long term exposure of flubendiamide through environmental source.

4. Conclusion

Result of the present study concluded that this extraction less HPLC method is very simple, reliable, repeatable and suitable for flubendiamide estimation in blood.

5. Acknowledgement

The authors are thankful to the Dean, College of Veterinary Science and Animal Husbandry, and Vice Chancellor of the University for providing the necessary laboratories facilities established in the Department of Pharmacology and Toxicology under Niche Area of Excellence Programme of ICAR and Rashtriya Krishi Vikas Yojana of Govt. of India. Routine financial assistance provided by the University for Doctoral Research to the first author is also duly acknowledged.

6. Conflict of Interest

There is no potential conflict of interest.

7. Competing Interest and Funding Statements

The work reported in this publication was a part of PhD thesis of first author. This research received no specific grant from any funding agency in the public, commercial, private, or not-for-profit sectors. The research work was undertaken with the routine facilities and grants available in the Department.

8. References

1. Government of India, Ministry of Agriculture, Department of Agriculture & Cooperation. Directorate of Plant Protection, Quarantine & Storage, Central Insecticide Board & Registration Committee, N.H. IV, Faridabad-121 001, Registered under the Insecticides Act, 1968 [Internet]. 2009. [cited 2015 Jan 1]. Available from: http://www.cibrc.nic.in/mupi.pdf

2. EPA (Environmental Protection Agency) [Internet]. 2010. [cited 2014 Oct 28]. Available from: http://www.gpo.gov/fdsys/pkg/FR-2010-11-05/html/2010-27998.html
3. Joint Meeting on Pesticide Residues (JMPR). Report of the joint meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO core assessment group on pesticide residues at the request of codex committee on pesticide residues Italy; 2010. p. 345–82.
4. Das SK, Mukherjee I, Roy A. Flubendiamide as new generation insecticide in plant toxicology: A policy paper. Advances in Clinical Toxicology. 2017; 2(2). https://doi.org/10.23880/ACT-16000122
5. Cavoski I, D'Orazio V, Caboni P, Miano TA. Spectroscopic study of possible mechanism of flubendiamide sorption onto humic acids. Geophysical Research Letters. 2009; 11: EGU2009–12819.
6. United States of Environmental Protection Agency (USEPA) [Internet]. 2010. [cited 28 Mar 2017]. Available from: http://www.gpo.gov/fdsys/pkg/FR-2010-11-05/html/2010-27998.html.
7. Agnihothri NP. Pesticide safety evaluation and monitoring. AICRP Pesticide Residue, Division of Agricultural Chemicals, IARI, New Delhi; 1999.
8. Mohapatra S, Ahuja AK, Deepa M, Jagadish GK, Rashmi N, Sharma D. Development of an analytic method for analysis of flubendiamide, des-iodo flubendiamide and study of their residue persistence in tomato and soil. Journal of Environmental Science and Health, Part B. Pesticides, Food Contaminants, and Agricultural Wastes. 2011; 46:246–71. https://doi.org/10.1080/03601234.2011.540536. PMid:21462054
9. Das SK, Mukherjee I, Das SK. Dissipation of Flubendiamide in/on Okra [*Abelmoschus esculenta* (L.) Moench] Fruits. Bulletin of Environmental Contamination and Toxicology. 2012; 88:381–4. https://doi.org/10.1007/s00128-011-0491-9. PMid:22147083
10. European Food Safety Authority. Modification of the existing MRL for flubendiamide in strawberries. EFSA Journal. 2013; 11(10): 3403 https://doi.org/10.2903/j.efsa.2013.3403
11. Buddidathi R, Mohapatra S, Siddamallaiah L, Manikrao G, Hebbar SS. Dissipation pattern of flubendiamide residues on capsicum fruit (*Capsicum annuum* L.) under field and controlled environmental conditions. Journal of Environmental Science and Health, Part B. Pesticides, Food Contaminants, and Agricultural Wastes. 2016; 51:44–51. https://doi.org/10.1080/03601234.2015.1080496. PMid:26549520
12. Bayer AG. Report MR-202/03, Laboratory Project ID: P601030020, Leverkusen; 2003.
13. Gopal M, Mishra E. Analytical method for estimation of a new insecticide flubendiamide and its safety evaluation for usage in rice crop. Bull Environ Contam Toxicol. 2008; 81(4):360–4. https://doi.org/10.1007/s00128-008-9470-1. PMid:18587521
14. Battu RS, Singh B, Kooner R, Singh B. Simple and efficient method for estimation of residues of flubendiamide and its metabolites desi-odoflubendiamide. Journal of Agricultural and Food Chemistry. 2008; 56(7):2299–302. https://doi.org/10.1021/jf073081s. PMid:18321053
15. Sorg DA, Buckner B. A simple method for obtaining venous blood from small laboratory animals. Proceeding of the Society for Experimental Biology and Medicine. 1964; 115:1131–2. https://doi.org/10.3181/00379727-115-29134. PMid:14166577
16. Rao TN, Rao ST, Silpa G. Determination of lymecycline and tetracycline residues in bovine milk followed by matrix solid-phase dispersion coupled to high performance liquid chromatography with ultraviolet detection. World Journal of Pharmaceutical Research. 2012; 1:1281–90.
17. Motoba. K. Absorption, distribution, metabolism and excretion of radiolabele NNI-0001 following a single oral administration to male and female rats. Final report (Amended I). Project number: GB/01,03/0222, LSRC/M04/114A. Unpublished study prepared by Nihon Nohyaku., Co. Ltd.; 2005.