



## RESEARCH ARTICLE

### A NEW ANALYTICAL APPROACH FOR THE DETERMINATION OF TRIAFAMONE AND ITS METABOLITES RESIDUES IN WEAT PLANT

<sup>1</sup>Karri. Apprao, <sup>2,\*</sup>Surendra Babu, M. S., <sup>1</sup>Basaveswara Rao, M. V. and <sup>1</sup>Nageswara Rao, T.

<sup>1</sup>Department of Chemistry, Krishna University, Machilipatnam, AP India

<sup>2</sup>Department of Chemistry, GITAM University, Hyderabad, Telangana, India

#### ARTICLE INFO

##### Article History:

Received 26<sup>th</sup> June, 2016

Received in revised form

29<sup>th</sup> July, 2016

Accepted 22<sup>nd</sup> August, 2016

Published online 30<sup>th</sup> September, 2016

##### Key words:

Silica Gel,

Triafamone and its Metabolites,

HPLC and Weat Leaves.

#### ABSTRACT

A simple and high sensitive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with PDA detection for determination of triafamone and its metabolite residues. The evaluated parameters include the extracts by silica gel SPE cartridge using acetonitrile and 0.1% acetic acid solvents. The method was validated using weat leaves spiked with triafamone and its metabolites at different fortification levels (0.05 and 0.5 µg/g). Average recoveries (using each concentration six replicates) ranged 84-94%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.02-2.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.02µg/g and 0.05µg/g respectively. Finally the weat leave samples were re analyzed by HPLC.

Copyright©2016, Karri. Apprao, Surendra Babu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Karri. Apprao, Surendra Babu, M. S., Basaveswara Rao, M. V. and Nageswara Rao, T., 2016. "A new analytical approach for the determination of triafamone and its metabolites residues in weat plant" *International Journal of Current Research*, 8, (09), 38393-38397.

## INTRODUCTION

Herbicide(s), also commonly known as weed killers, are chemical substances used to control unwanted plants (Gangwar *et al.*, 2010). Selective herbicides control specific weed species, while leaving the desired crop relatively unharmed, while non-selective herbicides (sometimes called "total weed killers" in commercial products) can be used to clear waste ground, industrial and construction sites, railways and railway embankments as they kill all plant material with which they come into contact. Apart from selective/non-selective, other important distinctions include persistence (also known as residual action: how long the product stays in place and remains active), means of uptake (whether it is absorbed by above-ground foliage only, through the roots, or by other means), and mechanism of action (how it works). Historically, products such as common salt and other metal salts were used as herbicides, however these have gradually fallen out of favor and in some countries a number of these are banned due to their persistence in soil, and toxicity and groundwater contamination concerns.

Herbicides have also been used in warfare and conflict. Triafamone (genehmigte ISO Bezeichnung) ist ein neues Herbizid aus der Gruppe der Sulfonamide (S. Deivasigamani *et al.*, 2016), In the present study, the determination of triafamone and its metabolites residues in weat leaf followed by solid phase extraction and new validated HPLC method. Various methods have been described for the determination of these residues, using solid-phase micro extraction (SPME) Supercritical fluid extraction (SFE) and liquid – liquid extraction( Muccio AD *et al.*, 2006; Steven J. Lehotay *et al.*, 2000). However, none of the published researches to date have reported the residue analysis of triafamone and its metabolites in weat leaf.

## Experimental

### Standards, Reagents and samples

The analytical standard of Triafamone (98.7%) and its metabolites AE 1887196 oxazolidine-dione (96.2%) and triafamone dihydro (96.4%) were purchased from Sigma Aldrich. HPLC grade acetonitrile and water was purchased from rankem formic acid was supplied from Merck Limited and weat leaves were collected from local field.

\*Corresponding author: Surendra Babu, M. S.,

Department of Chemistry, GITAM University, Hyderabad, Telangana, India.

## Standard stock solutions

### Triafamone

A 10.46 mg of reference analytical standard of Triafamone was weighed in 10ml volumetric flask and the volume was brought upto the mark using acetonitrile.

### AE 1887196 oxazolidine-dione

A 10.12 mg of reference analytical standard of Metabolite-1 AE 1887196 oxazolidine-dione (BCS-CR-79349) was weighed in a 10 ml volumetric flask and the volume was brought upto the mark using acetonitrile.

### Triafamone dihydro

A 10.16 mg of reference analytical standard of Metabolite-2 Triafamone dihydro (BCS AA10030) was weighed in a 10 ml volumetric flask and the volume was brought upto the mark using acetonitrile.

## Preparation of Calibration solutions

### Triafamone and its Metabolites

Different known concentrations of Triafamone, AE 1887196 oxazolidine-dione and Triafamone dihydro (0.02 - 2 mg/L) in acetonitrile were prepared by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient.

## Extraction procedure for wheat leaf samples

### Crop Matrix Preparation

A 50 g of dry ice was added into a homogenizer apparatus. A 50 g of the wheat sample was added to the apparatus in small portions. The sample was blended after each addition until a homogeneous mixture is obtained. The sample was poured in to the double plastic bags or other suitable container. The sample was stored in a freezer until the last traces of dry ice have sublimed. The bag was sealed and stored in frozen conditions.

### Extraction of Crop Matrix Samples

A 25 g of wheat sample was weighed into a 50 mL extraction bottle. A 5 mL of water was added in to the extraction bottle and mixed well. Allowed the sample to sit for 1 hour to hydrate. A 20 mL of acetonitrile was added and homogenize the sample. Transferred the 2.5 mL of the upper layer into a glass tube and diluted with 7.5 mL of water.

### Sample clean-up

The SPE silica cartridge was conditioned by rinsing the cartridge with 3 mL of acetonitrile followed by 3 mL of water. The extracted eluents was transferred into a SPE cartridge.

Allowed the residual liquid through the cartridge with a vacuum. A 3 mL of acetonitrile was used to elute the residues from the SPE cartridge. The eluent was transferred in to a round bottom flask and evaporated using a rotary evaporator at 45°C under a stream of nitrogen. A 2 ml of acetonitrile was added to the round bottom flask and mixed well. A 8 mL of 0.1% acetic acid and mixed well. The samples were injected into a HPLC.

## Instrumentation

### HPLC-PDA separation parameters

**Instrument:** Shimadzu High Performance Liquid Chromatograph system equipped with LC-20 AT pump and SPD-20A, PDA detector, with LC solution software

**Column:** XBridge C8 column (15cm length x 4.6mm i.d X5.0 µm) particle size

**Mobile Phase A:** Acetonitrile

**Mobile phase B:** 0.1% acetic Acid in Milli-Q water

**Flow (mL/min):** 1.2 mL per minute

Flow (mL/min):	1.0 mL per m	Time (min)	A Conc (%)	B Conc-(%)
0.01			45	55
5.0			45	55
10.0			85	55
15.0			85	55
18.0			45	55

**Flow program:** Injection volume: 20 µL.

**Detector:** PDA

**Detector wavelength:** 260 nm

Compound Name Retention time in minutes (Approximately)  
 Triafamone 11.2 AE 1887196 oxazolidine-dione 6.4 and 7.2  
 Triafamone dihydro 8.2 and 9.7

## 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 (Tentu Nageswara Rao *et al.*, 2015). The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 µg/g. Linearity was determined by different known concentrations (0.02, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD µg/g) 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/g) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

## RESULTS AND DISCUSSION

### Specificity

Aliquots of triafamone and its metabolites, control sample solution, 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).

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

### Detection and Quantification Limits

The limit of quantification was determined to be 0.05 µg/g. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (84-94%, RSD<2%) were achieved.

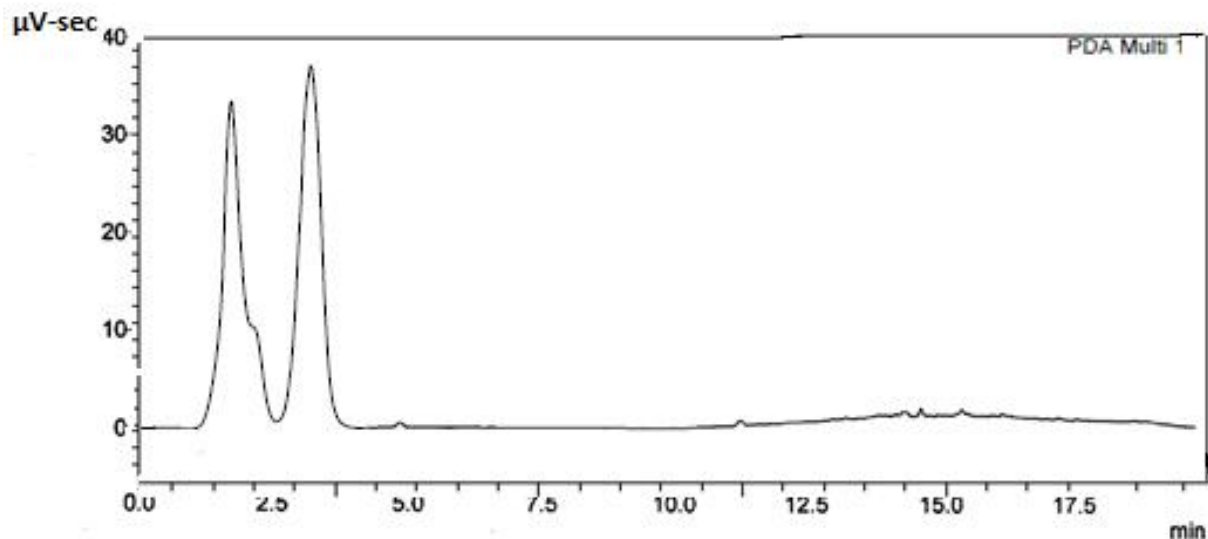


Figure 1. Representative Chromatogram at weat leaf control

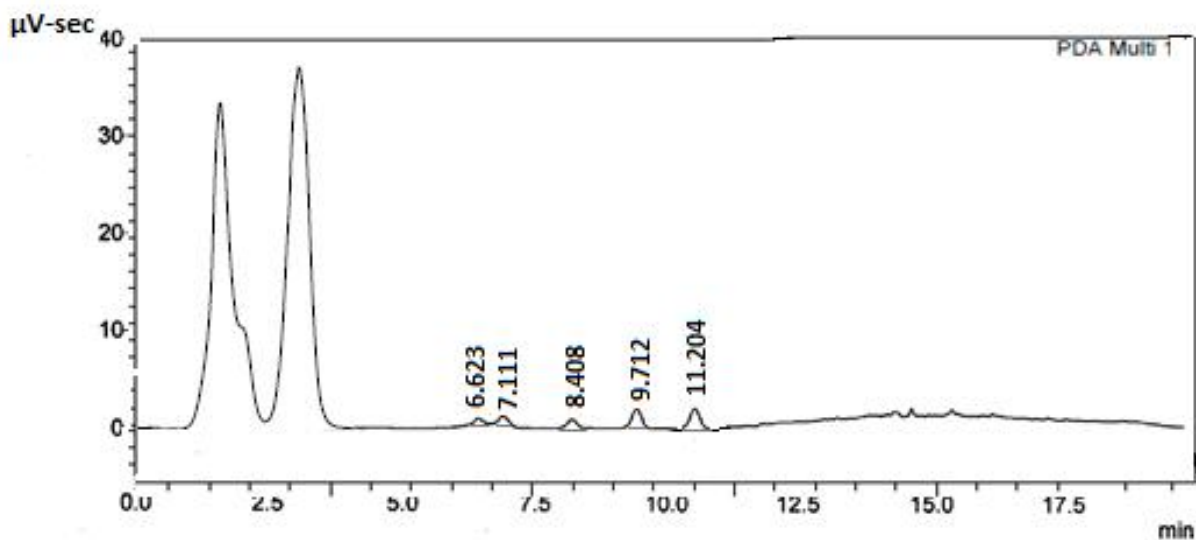


Figure 2. Representative Chromatogram at fortification level of 0.05 µg/g

Table 1. Calibration details

Concentration (mg/L)	Peak area (in µv-sec)		
	Triafamone	AE 1887196 oxazolidine-dione	Triafamone dihydro
0.02	214	187	174
0.05	524	447	412
0.1	1087	912	809
0.5	5248	4521	4021
1	10489	9047	8265
2	20874	18087	16387

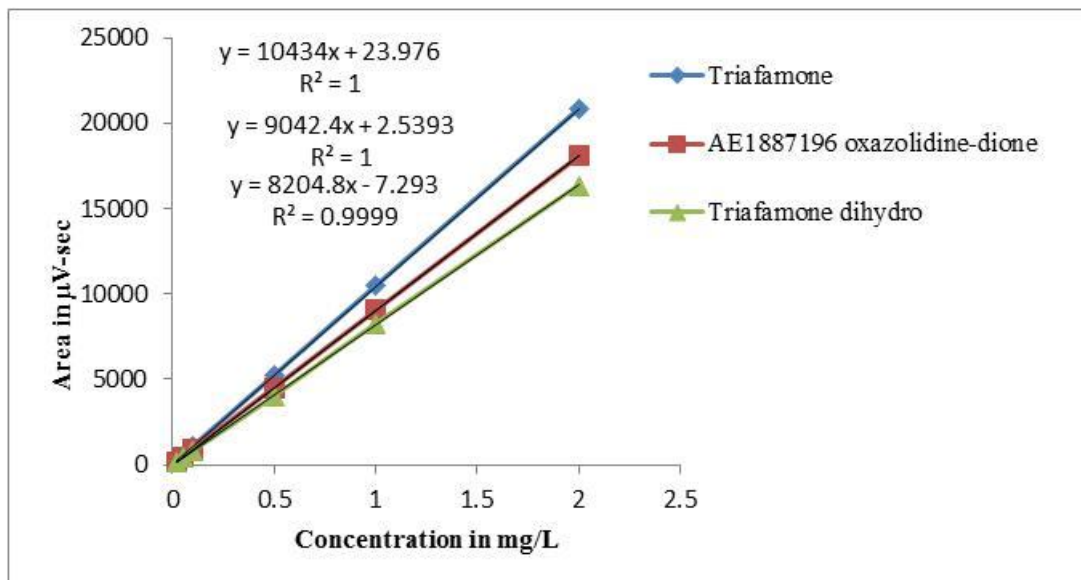


Figure 3. Representative Calibration curve of Triafamone and its metabolites

Table 2. Recoveries of the triafamone and its metabolites from fortified weat leaf control sample (n=6)

Fortification Concentration in μg/g	Replication	Recovery (%)		
		Triafamone	AE 1887196 oxazolidine-dione	Triafamone dihydro
0.05	R1	86	85	86
	R2	85	85	82
	R3	87	84	85
	R4	86	85	83
	R5	88	86	84
	R6	86	86	86
	Mean	86.33	85.17	84.33
	STDEV	1.03	0.75	1.63
	RSD in %	1.20	0.88	1.94
0.5	R1	94	91	90
	R2	95	90	89
	R3	94	93	92
	R4	93	92	93
	R5	93	94	91
	R6	95	95	92
	Mean	94.00	92.50	91.17
	STDEV	0.89	1.87	1.47
	RSD in %	0.95	2.02	1.61

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 0.05 μg/g at a level of approximately three times the background of control injection around the retention time of the peak of interest.

**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 (μV-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:

Recovered concentration or Dose concentration

$$= \frac{(x-c) \times D \times 100}{m \times P}$$

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

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

### Conclusion

This paper describes a fast, simple sensitive analytical method based on HPLC to determine the triafamone and its metabolites residues in weat leaf. The SPE extraction procedure is very simple and inexpensive method for determination of triafamone and its metabolites residues in weat. The mobile phase Acetonitrile and HPLC grade water showed good separation and resolution and the analysis time required for the chromatographic determination of the weat leaf is very short (around 20 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and LOQ were established by following South African National Civic Organization guidelines (SANCO Guidelines, 2009). Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the triafamone and its metabolites residues in different commodities (fruit, juice, seed, oil, and water and soil samples).

### Acknowledgement

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

### REFERENCES

- Deivasigamani, S. 2016. Bio-efficacy and phytotoxicity of triafamone and ethoxysulfuron in transplanted rice (*oryza sativa*). *International Journal of Multidisciplinary Research and Modern Education*, 2: 274-279.
- Gangwar, K.S. and H.R. Singh. 2010. Effect of rice crop establishment technique on succeeding crops. *Indian J. Agric Sciences*, 80 (1): 24.
- Karri, Apparao., Surendra Babu, MS., Basaveswara Rao, MV., Tentu. Nageswara Rao. 2015. A new method for determination of tolfenpyrad residues in mango fruit. *The Experiment*, 32: 2040-2047.
- Muccio AD, *et al.* 2006. 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: 1-6.
- SANCO Guidelines 2009. Method validation and quality control procedures for pesticide.
- Tentu Nageswara Rao., SNVS Murthy., S. Seshamma., D. Sreenivasulu. 2015. Spectrometry. *Journal of AOAC International*, 83 (3): 5.
- Steven J. Lehotay. 2000. Analysis of pesticide residues in mixed fruit and vegetable extracts by direct sample introduction/GasChromatography/Tandem Mass Independent Laboratory method validation and determination of spiromesifen residues in tomatos. *International Journal of Chem Tech Research*, 8 (3): 1149-1155.

\*\*\*\*\*