ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 06, pp.32352-32355, June, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SIMULTANEOUS DETERMINATION OF THIOPHANATE-METHYL AND ITS METABOLITECARBENDAZIM IN OKRA FRUIT

***,[1]Tentu. Nageswara Rao, [1]Krishnarao, N., [2]Parameshwar, K. [1]Karri. Apparao and [3]Prashanthi, Y.**

[1]Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India
[2]Department of Pharmacy, Princeton College of Pharmacy, Ghatkesar, Telangana, India
[3]Department of Chemistry, Mahatma Gandhi University,Nalgonda, Telangana, India

ARTICLE INFO

Article History:
Received 19th March, 2016
Received in revised form
21st April, 2016
Accepted 27th May, 2016
Published online 15th June, 2016

Key words:
HPLC-UV,
Thiophanate-Methyl,
Carbendazim and Okra fruit.

ABSTRACT

A easy, sensitive and inexpensive method was evolved the usage of solid-phase extraction, together with high performance liquid chromatographic method with UV detection for determination Thiophanate-Methyl and Its Metabolite Carbendazim residues in okra fruit samples. The evaluated parameters consist of the extracts via C_{18} cartridge, using acetonitrile solvent. The method becomes established the use of okra fruit samples spiked with Thiophanate-Methyl and Its Metabolite Carbendazim at different fortification levels (0.01 and 0.1 µg/g). Average recoveries (using each concentration six replicates) ranged 84-94%, with relative standard deviations less than 3%, calibration solutions concentration in the range 0.01-5.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.003µg/mL and 0.01µg/mL respectively.

Citation: Tentu. Nageswara Rao, Krishnarao, N., Parameshwar, K. Karri. Apparao and Prashanthi, Y. 2016. "Simultaneous determination of Thiophanate-Methyl and its Metabolitecarbendazim in okra fruit", *International Journal of Current Research*, 8, (06), 32352-32355.

INTRODUCTION

Thiophanate-methyl is a fungicide which belongs to the group of the benzimidazoles. It has been applied since the 1960s against a variety of fungal pathogens (Na Liua *et al*., 2014). In Germany it has been approved for control of white mould in winter rape (Sclerotinia sclerotiorum), fungal rots in stores of stone fruit and admitted under the trade name "Don-Q" for Fusarium head blight control in wheat and triticale since 2009 (Chen *et al*., 2013; Munetomo Nakamura, 2011). The primary effect of TM is caused by the transformation product methyl-benzimidazole-2yl-carbamate (MBC), which binds to the fungal β-tubulin and disturbs the formation of the spindle apparatus and cell division (Ono *et al*., 1975; Miyamoto *et al.,* 1996). Thiophanate-methyl and a number of metabolites could be liberated enzymatically or by acid treatment from water soluble conjugates in rat urine in the same studies. Identified compounds were thiophanate-methyl, 4-OH-TM, 4-OH-FH-432, FH-432, 5-OH-MBC and MBC (ZHANG Zhiyong *et al.*, 2012; Sudeb Mandal *et al*., 2010). As was the case in faces, the two of these compounds, namely MBC and 5-OH-MBC, may possibly have been formed during the analytical procedures (Jiao *et al.,* 2014).

****Corresponding author: Tentu. Nageswara Rao,***
Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India.

Diverse methods had been defined for the determination of those residues, the usage of stable-phase micro extraction (SPME) Supercritical fluid extraction (SFE) and liquid – liquid extraction. However, not one of the posted researches so far has reported the simultaneous analysis of Thiophanate-Methyl and Carbendazim in okra fruit.

Experimental

Standards, Reagents and samples

The analytical standards of Thiophanate-Methyl and Its Metabolite Carbendazim was obtained from Sigma Aldrich. HPLC grade acetonitrile, Formic acid and water became bought from Rankem, Analytical grade chemicals i.e., magnesium sulphate and sodium chloride were brought from Merck Limited and okra fruit become bought from local market.

Standard stock solutions

Accurately weighed 9.58 mg of reference analytical standard of Thiophanate-Methyl in 10ml volumetric flask and the volume was brought upto the mark using acetonitrile. A 10.12 mg of reference analytical standard of Carbendazim was weighed in a different 10ml volumetric flask and the volume was brought upto the mark using acetonitrile 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.

Sample preparation

Representative 25 gram portions of okra fruit fortified with 0.1 mL of working standard solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction procedure

Representative sample was (Okra 25g) homogenized and directly extracted with 50 ml of acetonitrile, after addition of magnesium sulphate, sodium chloride and buffering citrate salts (pH 5-5.5). The mixture was shaken intensively and centrifuged for phase separation. Evaporated the acetonitrile to 2 – 3 ml by using rotary vacuum evaporator. An aliquot of extract was cleaned up by C18 cartridge with magnesium sulphate. Final extracts were diluted with mobile phase for HPLC-UV determination of Thiophanate-methyl, its metabolite Carbendazim.

Chromatographic separation parameters

Instrument : Shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software

Detector : UV detector

Column : Phenomenex luna C18 (250 mm length x 4.6mm I.D x 5µ particle size)

Mobile Phase: A = Acetonitrile
B = 0.1% Formic acid

Time (min)	% A	% B
0	50	50
4	90	10
10	90	90
15	50	50

Flow Rate: 0.5 mL/min
Column Temperature: 30°C
Wave Length: 254 nm
Injection Volume: 20 µL

Retention time (approximate)

Thiophanate-methyl- 8.9 minutes
Carbendizm (MBC)- 5.3 minutes

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 (Karri. Apparao *et al.*, 2015). The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.01 and 0.1 µg/mL. Linearity was determined by different known concentrations (0.01, 0.05 0.1, 0.5, 1.0 and 5.0 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 (SANCO Guidelines, 2009).

RESULTS AND DISCUSSION

Specificity

Aliquots of Thiophanate-methyl Carbendizm samples, 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). Furthermore, the retention time of Thiophanate-methyl Carbendizm were 8.9 min and 5.3 min (Approximately).

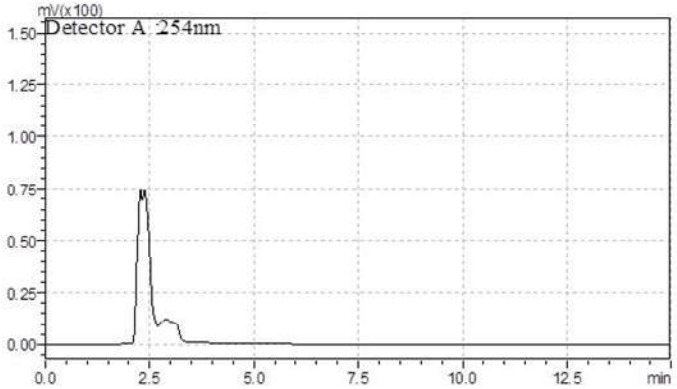

Figure 1. Representative Chromatogram at okra fruit control

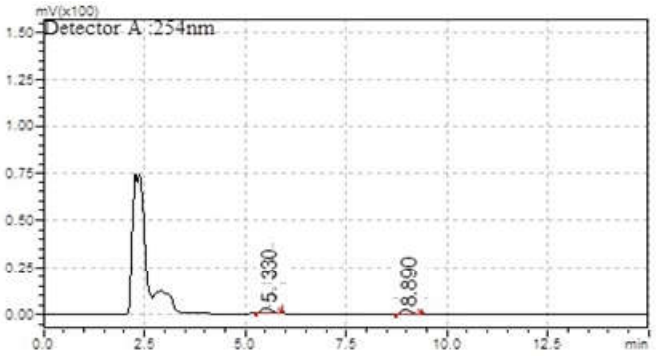

Figure 2. Representative Chromatogram at fortification level of 0.01 µg/mL

Calibration Details

Preparation of Stock solution of reference analytical standard

Accurately weighed 9.58 mg of reference analytical standard of Thiophanate-methyl in 10ml volumetric flask and the volume was brought upto the mark using acetonitrile. A 10.12 mg of reference analytical standard of Carbendizm was

weighed in a different 10ml volumetric flask and the volume was brought upto the mark using acetonitrile.

Preparation of Calibration solutions

Mixture of different known concentrations of Thiophanate-methyl and Carbendizm (5 - 0.01 µg/mL) were prepared in 10ml acetonitrile 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. Results are presented in Table 1. Calibration curve was presented in (Figure 3).

Table 1. Serial dilutions of linearity standard solutions

Concentration (µg/g)	Peak area (mAU)	
	Thiophanate-methyl	Metabolite Carbendazim (MBC)
5	123054	101741
2	49506	41892
1.0	23987	20036
0.5	12832	10023
0.1	2354	2054
0.01	234	210

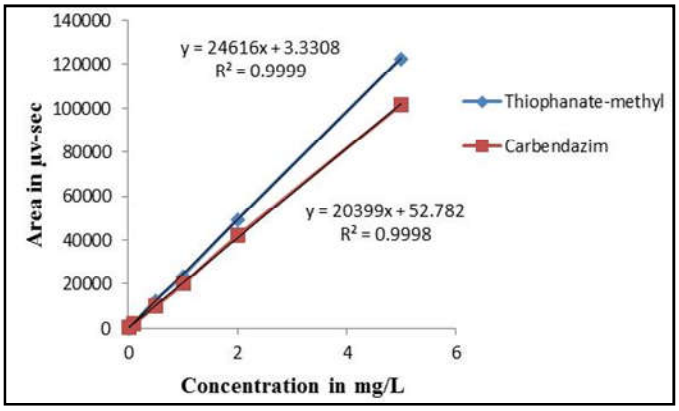

Figure 3. Representative Calibration Curve of Thiophanate-methyl and Carbendizm

Recovery-Limit of Determination (LOQ)

Recovery studies in okra fruit was conducted by fortifying different concentrations of Thiophanate-methyl and Carbendizm standards in the range (0.01 - 0.1 µg/mL). The samples were homogenized, extracted and analysed for residue content, as described in the method of analysis. The average percent recovery for Thiophanate-methyl in okra fruit was 86±1.80 at 0.01 µg/mL fortification level and 94±1.57 at 0.1 µg/mL fortification levels, respectively. The Carbendizm has the recovery percentage 84±1.45 at 0.01 µg/mL fortification level and 93±1.14 at 0.1 µg/mL fortification levels, respectively in okra fruit. The method has a limit of determination 0.01 µg/mL (LOQ).

Storage Stability

A storage stability study was conducted at -20°C with Soyabean samples spiked with 0.1 µg/ml of Thiophanate-methyl and Carbendizm Samples were stored for a period of 30 days at this temperature. Analysed for the content of Thiophanate-methyl and Carbendizm before storing and at the end of storage period. The percentage of dissipation observed during above storage period was only 4% showing for both Thiophanate-methyl and Carbendizm no significant loss of residues on storage. Results are presented in Table 2.

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:

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

Where,

m = the slope of the line from the calibration curve
x = sample area of injected sample (µV*sec)
c = 'y' intercept of the calibration curve
D = Dilution Factor
P = Purity of Test item

Table 2. Storage stability Details

Fortified concentration (µg/mL)	Storage Period (days)	Replication	Recovery (%)	
			Thiophanate-methyl	Carbendizm
0.1	0	R1	94	90
		R2	92	91
		R3	91	93
		Mean ± S.D	92±1.65	91±1.67
	30	R1	90	89
		R2	89	90
		R3	90	88
		Mean ± S.D	90±0.64	89±1.12

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

Conclusion

This paper describes a fast, simple sensitive analytical method based on HPLC-UV to determine the Thiophanate-methyl and

Carbendizm residues in okra fruit. The SPE extraction procedure is very simple and inexpensive method for determination Thiophanate-methyl and Carbendizm residues in okra fruit. The mobile phase Acetonitrile and 0.1% Formic acid showed good separation and resolution and the analysis time required for the chromatographic determination of the sugarcane juice is very short (around 15 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and LOQ were established by following South African National Civic Organization (SANCO) guidelines Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine Thiophanate-methyl and Carbendizm residues in different commodities (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

Chen, H., Liu, X., Wang, C., Wang, Q., Jiang, Y., Yin, P., Zhu, L. 2013. Simultaneous Determination of Thiophanate-Methyl and Its Metabolite Carbendazim in Tea Using Isotope Dilution Ultra Performance Liquid Chromatography–Tandem Mass Spectrometry. *Journal of Chromatographic Science*, 52: 1157–1164.

Jiao, L., Dong, D., Zheng, W., Zhang, S., Shen, C. 2014. Determination of thiophanate-methyl using UV absorption spectra based on multiple linear regression. *Optik*, 125: 183–185.

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.

Miyamoto, F., Saeki, M. 1996. Determination of thiophanate methyl in agricultural products by high performance liquid chromatography. *Chiba Eiken Hokoku*, 20: 12 -17.

Munetomo nakamura. 2011. Determination of Carbendazim, Thiophanate, Thiophanate-methyl and Benomyl Residues in Agricultural Products by Liquid Chromatography-Tandem Mass Spectrometry. *Food Hygiene and Safety Science*, 52:148-155.

Na Liua., Fengshou Dong. 2014. Effect of household canning on the distribution and reduction of thiophanate-methyl and its metabolite carbendazim residues in tomato. *Food control*, 43: 115–120.

Ono, S., Hasegawa, J., Yanase, R., Sugioka, K., Tohyama, N. 1975. Residue analysis of thiophanate methyl and its degradation product by ultra violet absorption spectroscopy. *Bunseki Kagaku*, 24:750 - 754.

SANCO Guidelines. 2009. Method validation and quality control procedures for pesticide residues analysis in food and feed. *Document NO. SANCO/10684/2009*.

Sudeb Mandal., Saktipada Das., Anjan Bhattacharyya. 2010. Dissipation Study of Thiophanate Methyl Residue in/on Grapes (*Vitis vinifera* L.) in India. *Bulletin of Environmental Contamination and Toxicology*, 84: 592-595.

ZHANG Zhiyong., GONG Yong., SHAN Weili., JIAN Qiu., SHEN Yan., LIU Xianjin. 2012. Determination of thiophanate-methyl and carbendazim in cucumber and soil by QuEChERS-high performance liquid chromatography-triple quadrupole tandem mass spectrometry. *Chinese Journal of Chromatography,* 30: 91-94.

****