ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND OPTIMIZATION FOR THE DETERMINATION OF TRIAZOLE FUNGICIDES BY GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Abstract

Tebuconazole is a Triazole fungicide used agriculturally to treat plan pathogenic fungi. It is widely use for apple and grapes. Technical grade of tebuconazole was collected from shufalan crop science. Stock solution of 2500 ppm made by acetone, tebuconazole and pulp of apple. Test solution of 500 ppm, 1500 ppm, 2500 ppm, 3500 ppm made for analysis in SHIMADZU GC-MS QP 2010 ULTRA equipped with single quadrupole mass detector. 1 micro litre of sample used in every analysis. In linearity test five concentration sample were injected and the graph of area versus concentration found linear. The sample of 2500 ppm which was injected five times for precision study the area (12648071) and retention time (RT-9.38) found same for individual injection. Taken the 500 ppm, 2500 ppm, and 4500 ppm of sample set as three sample of each for accuracy test and in the result the average of area and retention time were accurate for individual sample set. Tebuconazole is highly toxic for human body therefore FSSAI decided maximum residue limit 1 ppm. The method of GC-MS developed to find out the quantity of tebuconazole present in apple. The limit of quantification of the method was 30 ppm and the limit of detection was 10 ppm.

Keyword

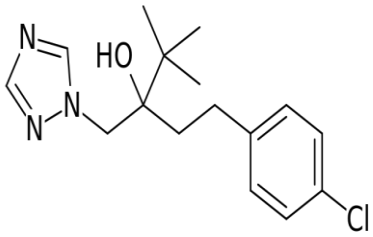
GC-MS, tebuconazole, fungicide.

INTRODUCTION

Tebuconazole

- Tebuconazole is a Triazole fungicide insecticide. It was introduced in 1986 first, Reported in 1988 By South Africa. Though the U.S. Food and Drug Administration considers this fungicide to be safe for humans, it may still pose a risk. It is listed as a possible carcinogen in the United States Environmental Protection Agency Office of Pesticide Programs carcinogen list with a rating of C (possible carcinogen). According to the World Health Organization.

- Toxicity classification, it is listed as III, which means slightly hazardous.
- Tebuconazole is uses as a symmetric action that can work to prevent and eradicate fungi. This chemical compound can eliminate fungi by inhibiting their ability to spread spores, which slows growth.



- IUPAC NAME-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H, 1,2,4-triazol-1- ylmethyl) pentan-3-ol
- CHEMICAL FORMULA – C16H22ClN3O
- MOLAR MASS – 307.822 g/mol
- MELTING POINT – 102.4˚C

MATERIAL AND METHODS

Solvent and sorbent

- Acetone (HPLC grade) from SPECTROCHEM
- Sodium sulfate anhydrous – Na_2So_4 (LR grade) from MERCK

Apparatus

SHIMADZU GCMS-QP2010 ULTRA Equipped with single quadrupole mass detector.99.99% pure Nitrogen gas cylinder. REMI R-8C DX homogenizer was used for sample homogenization. Rtx-OPPesticides Column using. 10(µl) GC injector are using to sampling.

Preparation of standard solution

Take 10gm of apple containing 50mg of triazole fungicide (tebuconazole),add 10ml solvent (acetone), 4gm sorbent Sodium sulfate anhydrous - Na_2So_4 and shak continusly for 1 min. after shaking centrifuge the mixture at 4500 RPM for 15 min. andcollect the layer from the upper surface of centrifuge tube.

Preparation of test solution

1) **20% Solution:** From stock solution of 5000 ppm 0.1ml was taken into 1 ml Eppendorf and diluted up to 1ml with diluent (Methanol & Acetone) to get 500 ppm fungicide solution.
2) **60% Solution:** From stock solution of 5000 ppm 0.3ml was taken into 1 ml Eppendorf and diluted up to 1ml with diluents (Methanol & Acetone) to get 1500 ppm fungicide solution.
3) **100% solution:** From stock solution of 5000 ppm 0.5ml was taken into 1 ml Eppendorf and diluted up to 1ml with Acetone to get 2500ppm fungicide solution
4) **140% Solution:** From stock solution of 5000 ppm 0.7ml was taken into 1 ml Eppendorf and diluted up to 1ml with diluents (Methanol & Acetone) to get 3500 ppm fungicide solution.
5) **180% Solution:** From stock solution of 5000 ppm 0.9ml was taken into 1 ml Eppendorf and diluted up to 1ml with diluents (Methanol & Acetone) to get 4500 ppm fungicide solution.

Method parameters of GC-MS

GC-MS parameters			
GC		**MS**	
Injection Mode	Split	Ionization voltage	70eV
Injection Temperature	260˚C	Detector voltage	0.8kV
Carrier Flow	89.7 cm/sec	Solvent cut time	2.5 min
Carrier Gas	Helium	Interface temperature	310˚C
Purge Flow	3.5 ml/min	Ion source temperature	200˚C

Split Ratio	10	Mode	Scan
Column oven		Scan range	m/z to 999 m/z
Initial	100 ˚C		
Hold time	5.0 min	Scan speed	3333 sec
Ramp	20˚ C min^{-1}	Total time : 15 min	
Final	300 ˚C		
Hold time	5.0 min		

METHOD VALIDATION

Specificity

The specificity of the method was determined by checking the interference of diluents (Methanol & Acetone) which were used for sample preparation. There was no any peak of diluent affecting or interfering with the analytes peaks.

Chromatogram of Diluent Acetone

When Acetone was taken as a diluent, the RT was found at 1.5 min. which is not merged with any other sample peaks.

Linearity

The linearity was prepared with five concentration levels by injecting 1 µl of each concentration. The range of concentration levels were 500, 1500, 2500, 3500 & 4500 ppm. The response of the each concentration found to be linear in the investigation concentration range.

Summary of concentration for linearity (Apple extract) in Solvent

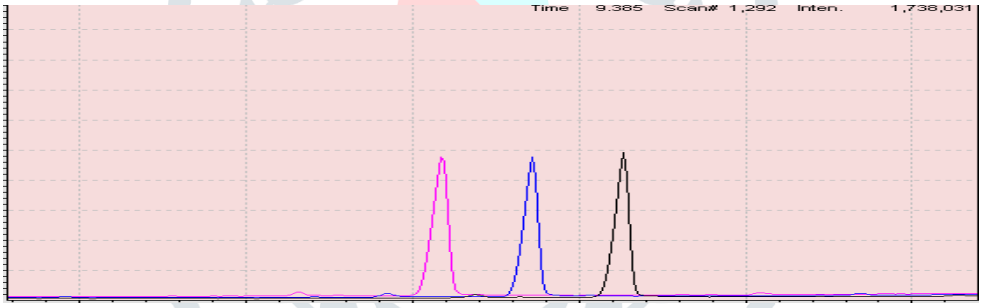
Sr. No.	Concentration Level	Total Volume (ml)	Fungicide Tebuconazole (mg)	Injection Volume (µl)	Concentration (ppm)
1	Level 1 (20%)	1	5	1	500
2	Level 2 (60%)	1	15		1500
3	Level 3 (100%)	1	25		2500
4	Level 4 (140%)	1	35		3500
5	Level 5 (180%)	1	45		4500

Accuracy

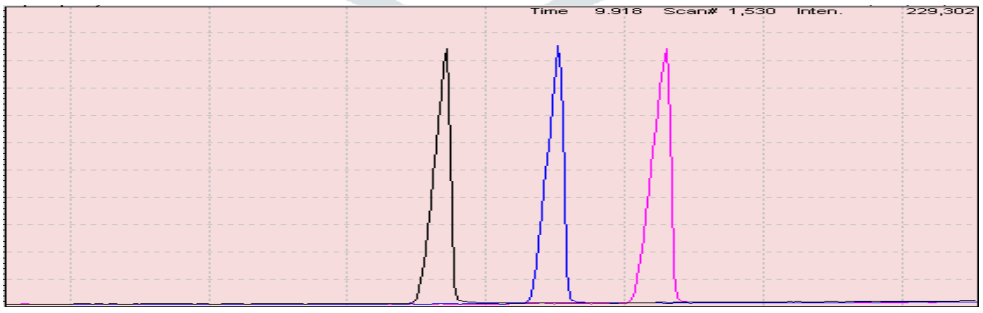
The accuracy of the method was assessed by determination of three concentrations for Tebuconazole (corresponding to 20%, 100% and 180% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected.

Summary of concentration for accuracy (Apple extract in Solvent).

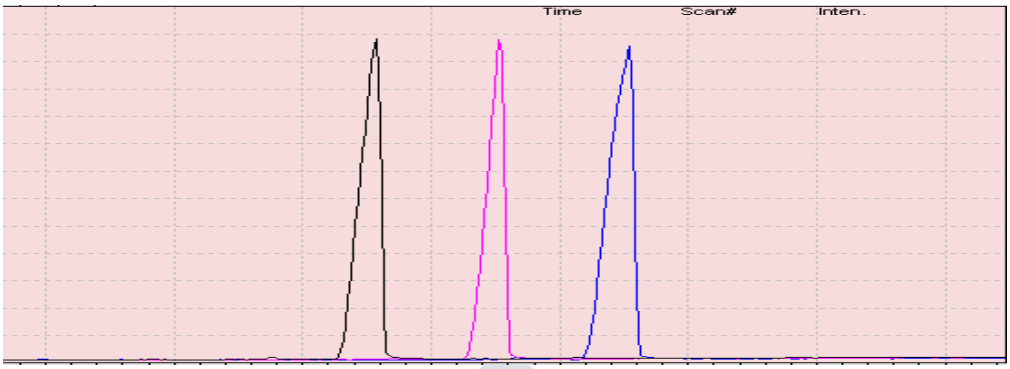
Sr. No.	Concentration Level	Total Volume (ml)	Tbuconazole (mg)	Injection Volume (µl)	Concentration (ppm)	Replicates
1	Level 1 (20%)	1	05	1	500	3
2	Level 2 (100%)	1	25	1	2500	3
3	Level 3 (180%)	1	45	1	4500	3



Overlay of Accuracy for Tebuconazole in Acetone (Level 1, 20%)



Overlay of Accuracy Tebuconazole in Acetone (Level 2, 100%).



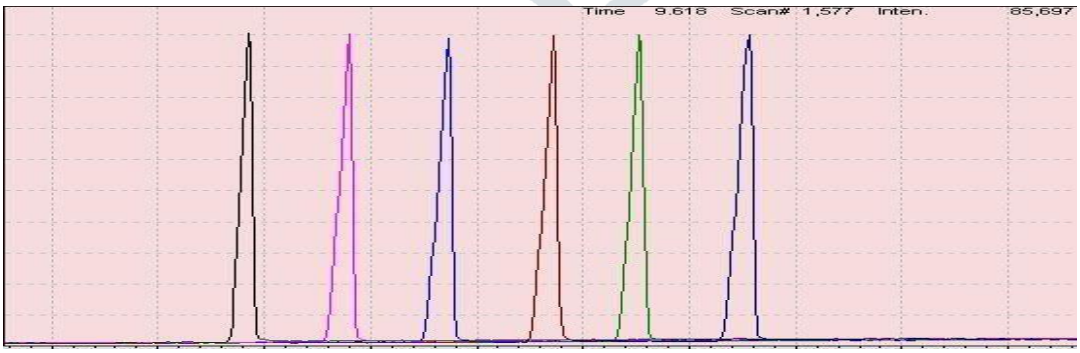
Overlay of Accuracy Tebuconazole in Acetone (Level 3, 180%).

Precision

The precision of the method was evaluated by carrying out six replicates of 100% test solution (2500 ppm) of triazole fungicide on the same day.

Summary of concentration for Precision study (Apple extract in Solvent, Same day).

Sr. No.	Concentration (%)	Total Volume (ml)	Tebuconazole (mg)	Injection Volume (µl)	Concentration (ppm)	Replicates
1	100	1	25	1	2500	6



Overlay of Precision Tebuconazole in Acetone (Level 100%).

LOD & LOQ

LOD and LOQ level obtained for this method for Tebuconazole fungicide is given in below table.

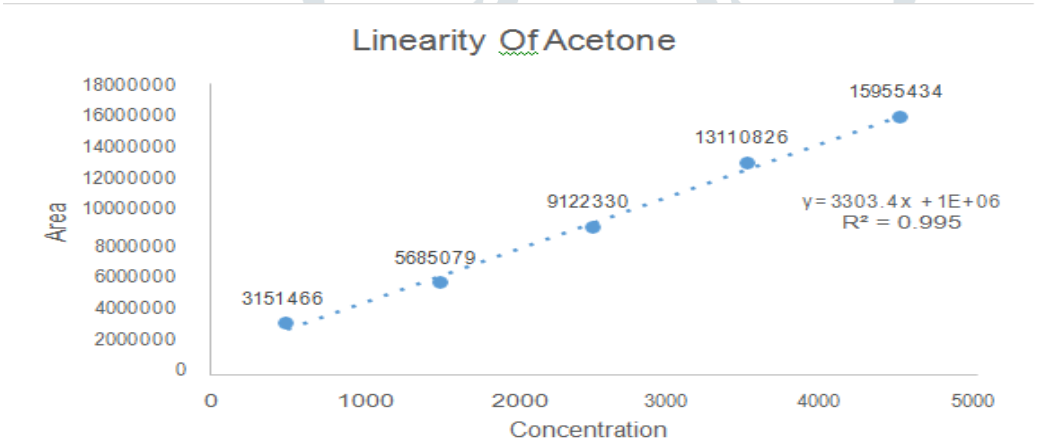
LOD and LOQ study of Triazole Fungicide

Sample	LOD (ppm)	LOQ (ppm)
Triazole Fungicide residue	10	30

RESULTS

Linearity Study of Triazole fungicides

Linearity Level	Triazole fungicides (Acetone)		
	Concentration (ppm)	AREA	RT
20%	500	3151466	9.36
60%	1500	5685079	9.37
100%	2500	9122330	9.37
140%	3500	13110826	9.38
180%	4500	15955434	9.40



Accuracy and precision study for Triazole fungicide

Solvent	Accuracy			Precision		
	Concentration Level	(Area) % RSD	(RT) % RSD	Concentration Level	(Area) % RSD	(RT) % RSD
Acetone	20 % (500 ppm)	1.7496717	0	100 % (2500 ppm)	2.85783	0
	100 % (2500 ppm)	1.6217201	0			
	180 % (4500 ppm)	1.8135919	0.061377			

The acceptance criteria for linearity is 0.99 R^2, for precision and accuracy is %RSD < 20% according to guideline. All above mentioned values for linearity, precision and accuracy comes under the acceptance criteria of guideline

Conclusion

The observation and upshots obtained from each validation experiment including specificity, linearity and range, LOD and LOQ, precision and accuracy lies well inside the acceptance criteria. Since, all the results are within the limit, the developed analytical method for Triazole Fungicide is considered as validated and suitable for projected use.

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Reference

1. Krynitsky, A. J. and Lehotay, S. J. 2002. "Overview of analytical technologies available to regulatory laboratories for the determination of pesticide residues". In Handbook of Residue Analytical Methods for Agrochemicals, Edited by: Lee, P. W. 753–786. Chichester,, England: Wiley & Sons.
2. Mills, P. A., Onley, J. H. and Guither, R. A. 1963. Rapid method for chlorinated.
3. Pesticide residues in nonfatty foods. J. Assoc. Off. Anal. Chem., 46: 186–191.
4. Hill, A. R. and Reynolds, S. L. 1999. Guidelines for in-house validation of analytical methods for pesticide residues in food and animal feeds. Analyst, 124(6): 953–958. [CrossRef], [Web of Science ®], [CSA]
5. Muhamad, H., Zainol, M., Sahid, I., & Seman, I. A. (2012). Determination of hexaconazole in field samples of an oil palm plantation. Drug Testing and Analysis, 4, 112–117. doi:10.1002/dta.1351
6. Nguyen, T. D., Han, E. M., Seo, M. S., Kim, S. R., Yun, M. Y., Lee, D. M., & Lee, G.-H. (2008). A multi-residue method for the determination of 203 pesticides in rice paddies using gas chromatography/mass spectrometry. Analytica Chimica Acta, 619(1), 67–74. doi:10.1016/j.aca.2008.03.031
7. Mladenova, R. I., & Shtereva, D. D. (2011). Multiresidue determination of pesticides by solid-phase extraction and GC-MS for control of peach production in Bulgaria. International Journal of Environmental Analytical Chemistry, 91(6), 567–575. doi:10.1080/03067310903108378
8. Alder, L., Greulich, K., Kempe, G. and Vieth, B. 2006. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS? Mass Spectrom. Rev., 25(6): 838–865.
9. Farajzadeh, M. A., Djozan, D., Mogaddam, M. R. A., & Bamorowat, M. (2011).Extraction and preconcentration technique for triazole pesticides from cow milk using dispersive liquid-liquid microextraction followed by GC-FID and GC-MS determinations.Journal of Separation Science, 34(11), 1309–1316. doi:10.1002/jssc.201000928