IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Development And Validation Of Effective Analytical Method For The Pesticide Formulation Combination Of Tebuconazole 0.3 % + Prothioconazole 1.54% + Metalaxyl 0.62 % By Reverse Phase HPLC

Chandrakant A Sonawane *, Dr. Chatur Pundlik Sawant

RFNS Senior College , Akkalkuwa , Kavayitri Bahinabai Chaudhari North Maharashtra University Jalgaon Maharashtra India

Abstract: Developed a simple precise, accurate, rapid, less analysis time consuming and economical reverse phase high performance liquid chromatography (HPLC) method and subsequently validated for the quantification of Tebuconazole + Prothioconazole + Metaxyl pesticide formulation using Inert Sustain ™ PFP (GL Science) 4 x100 mm, 5µm column Mixture of 0.1% ortho-phosphoric acid: Acetonitrile (50:50 v/v) was used as mobile phase The flow rate was kept 1.0 ml/min and detection was carried out at 220 nm. The limit of detection was 0.0001mg/ml, 0.0001mg/ml and 0.0001mg/ml for Metalaxyl, Prothioconazole and tebuconazole respectively. The limit of quantitation values was 0.001mg/ml, 0.001 mg/ml and 0.001mg/ml for Metalaxyl, Prothioconazole and tebuconazole respectively. The linearity of proposed method was investigated in be range of 0.30-0.90mg/ml(r^2=0.999),0.075-2.25mg/ml(r^2=0.999)and0.15-.45mg/ml(r^2=0.999) for Metalaxyl, Prothioconazole and tebuconazole respectively. The developed method was found to be specific, linear, precise, accurate ,robust ,analysis time saving and economical This combination is used for the seed treatment.

Keywords: Tebuconazole -Prothioconazole - Metaxyl pesticide formulation external standard validated method HPLC

Introduction:

Analytical methods may not be available for the pesticide in the form of a formulation due to the interference caused by the formulation excipients Analytical methods for a pesticide in specific combination with other pesticide may not be available. It may also involve some extraction and separation procedures, and these may not be reliable. Concentration of active ingredient the formulation is at very low level & achieving high level of precision & accuracy is quite difficult Improper detection of active content leads to poor efficacy, users may increase doses rates or the number of applications and unknowingly increase other risks If the concentration of these insecticides in a particular formulation exceeds the level claimed, its adverse effect may affect the crop and ultimately human health, whereas if the concentration is below the level claimed, the formulation is no longer effective for the crop. Therefore, it is necessary to analyses active accurately. Additionally, it is necessary to develop analytical methods that use fewer chemical reagents (i.e., that have low toxicity or low adverse health effects) and are more sensitive and reliable. A proposed analytical method should also address the issues of optimization, sensitivity, and reliability for quantitative determination of the target compounds in their respective formulations. Therefore, we developed and validated such an HPLC method—which is simple, rapid, precise, low-cost, and green for the simultaneous determination of different active material and formulated products

Method:

Materials

Certified Reference materials (CRM) of Metalaxyl, Prothioconazole and Tebuconazole were procured from Sigma Aldrich. The analytical grade working standards were prepared by two times crystallization of these technical grade materials. The analytical standards were qualified against CRMs and calculated purity as Metalaxyl (99.3%), Prothioconazole l (99.5%) and Tebuconazole (99.0%). These standards used for further analysis. Sample of Pesticide formulation for seed treatment fungicide containing Metalaxyl, 0.62 % w/w Prothioconazole 1.54% w/w and Tebuconazole 30% w/w was prepared in laboratory.HPLC grade acetonitrile was purchased from Qualigens Thermo Fisher Scientific, Mumbai (India). Mili-Q (Millipore India Pvt. Ltd) system used to generate HPLC grade water. Analytical grade Ortho-phosphoric acid (88%)

Apparatus :

A high-performance liquid chromatograph Shimadzu Prominence -I ,LC-2030 C equipped with PDA detector and software LC solution.

Chemicals

1. Acetonitrile (HPLC grade)
2. Water (HPLC grade)
3. Orthophosphoric Acid (H_3PO_4)

Diluent : Mobile phase use as diluent

HPLC Conditions :

A) Column : Inert Sustain ^{TM} PFP (GL Science) 4 x100 mm , 5µm

B) Mobile Phase : Acetonitrile: Water: Orthophosphoric Acid (40:60: 0.1 v/v)

C) Flow : 1.0 ml/min

D) UV wavelength : 220 nm

E) Injection volume : 10 ul

F) Column temperature : 30°C

G) Run time : 8 min

Preparation of Standard Standard Solution

The Standard stock solution is prepared in 50 ml volumetric flask by dissolving 62 mg of Metalaxyl (99.3%), 175 mg of Prothioconazole (99.5%) and 29.5 mg of tebuconazole (99.0%) dissolved in 25 ml acetonitrile then this solution is sonicate for 5 min, allow to cool at room temperature and make up to the mark with diluent.

Preparation of sample Solution :

Weight 500 mg of sample in 50 ml volumetric flask dissolved and sonicate for 5 min, allow to cool at room temperature and make up to the mark with diluent

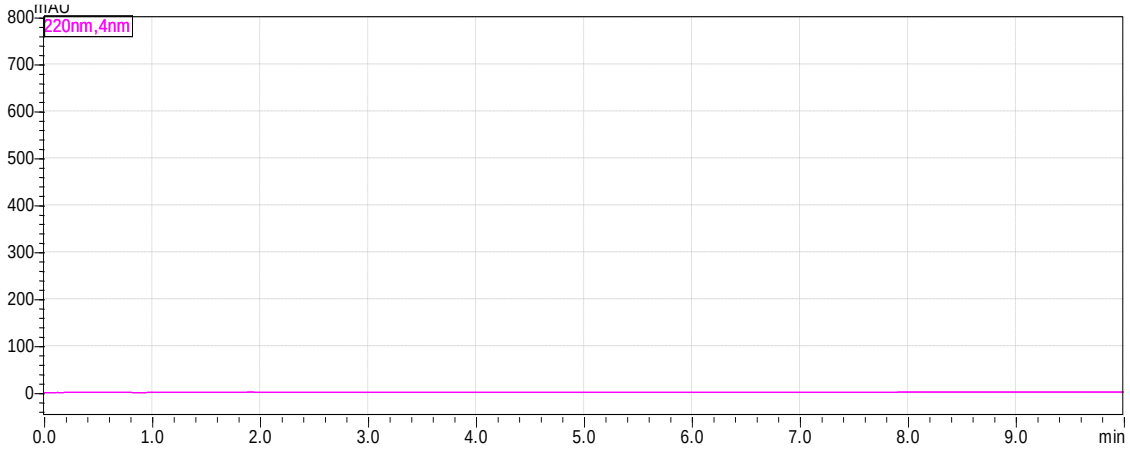
System Suitability

System suitability is very important, and it is performed to enhance the resolution of Chromatographic condition. It is observed that replicate injections of Metalaxyl , Prothioconazole and tebuconazole shows good precision conformed the suitability of proposed method.

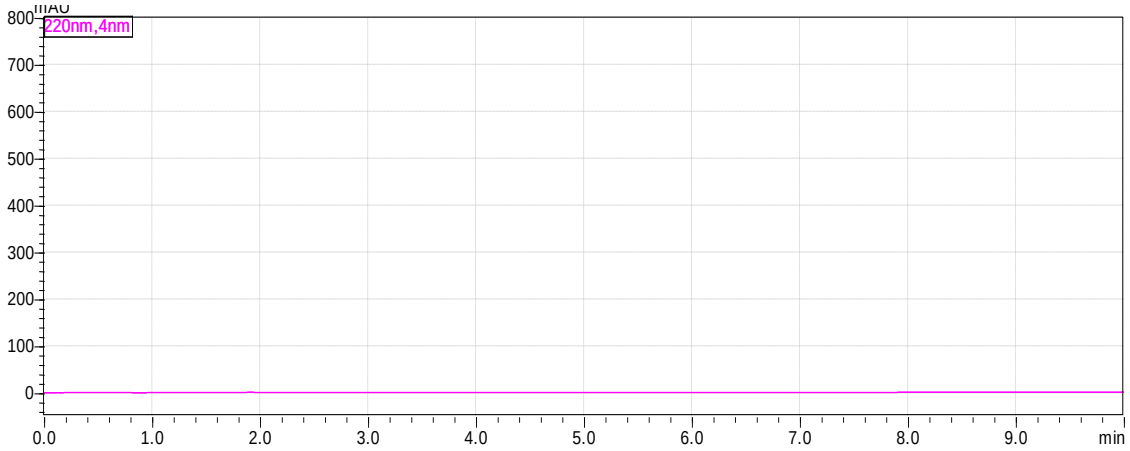
Parameters	Results			Limits
	Metalaxyl	Prothioconazole	Tebuconazole	
% RSD of retention time	0.12	0.11	0.11	< 1.0 %
% RSD of peak area	0.16	0.16	0.29	< 1.0 %

Specificity

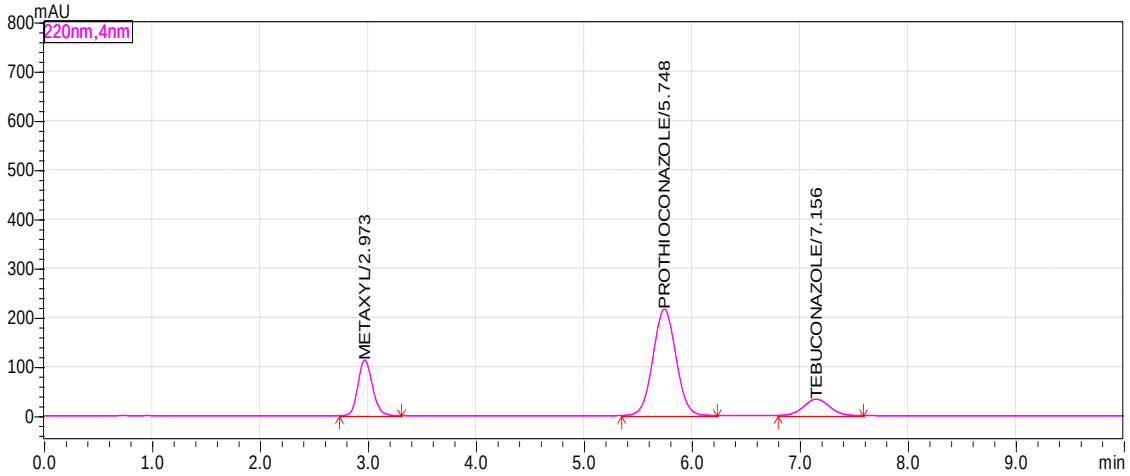
The specificity of the method will be determined by injecting individual solutions of reference standards of Metalaxyl, Prothioconazole and tebuconazole, reference standard mixture solution, test item sample solutions to find out the degree of interference with each other.



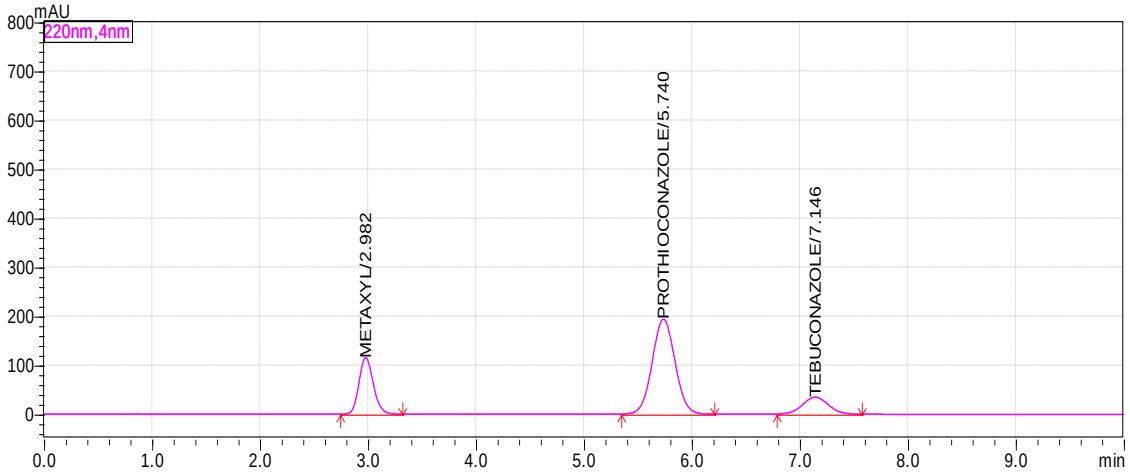
Diluent Blank Chromatograph



Formulation Blank Chromatograph



Standard Sample Chromatograph

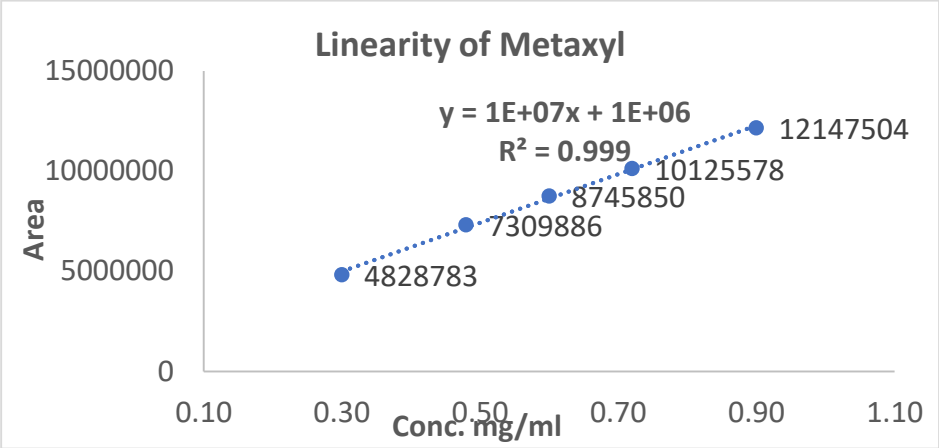


Sample Chromatograph

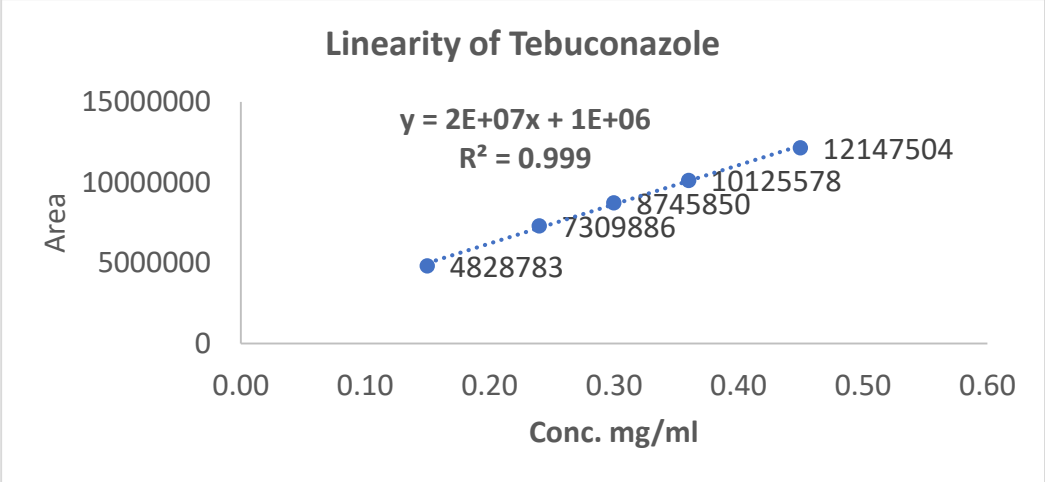
Linearity

Linearity curve will be established by analyzing five different concentrations level 50%, 80%, 100%, 120 % and 150% of standard solution of Metalaxyl, Prothioconazole and tebuconazole. The linearity curve plotted concentration of standard (mg/ml) against mean peak areas and the correlation coefficient value was computed

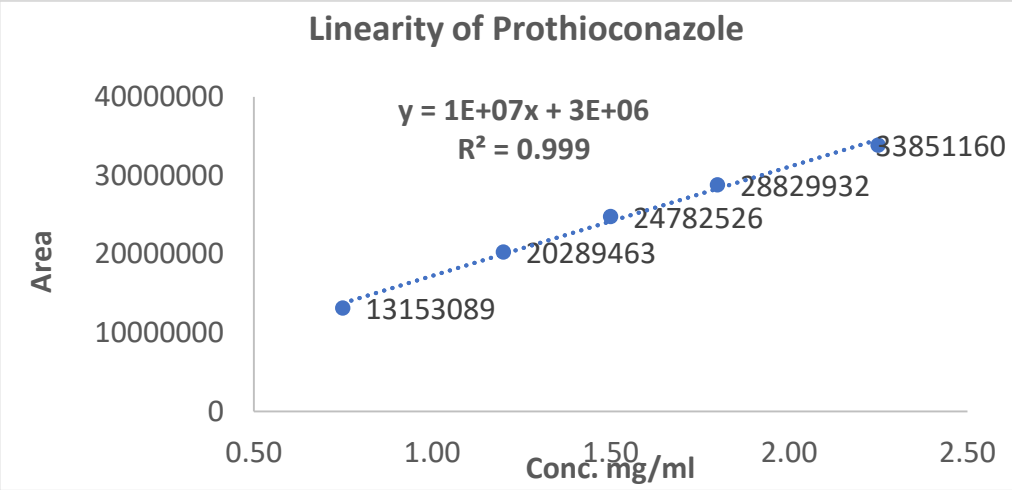
Linearity level	Concentration (mg/ml)	Tebuconazole Area
Level-1 (50%)	0.15	4828783
Level-2 (80%)	0.24	7309886
Level-3 (100%)	0.30	8745850
Level-4 (120%)	0.36	10125578
Level-5 (150%)	0.45	12147504



Linearity level	Concentration (mg/ml)	Metaxyl Area
Level-1 (50%)	0.30	4828783
Level-2 (80%)	0.48	7309886
Level-3 (100%)	0.60	8745850
Level-4 (120%)	0.72	10125578
Level-5 (150%)	0.90	12147504



Linearity level	Concentration (mg/ml)	Prothioconazole Area
Level-1 (50%)	0.75	13153089
Level-2 (80%)	1.20	20289463
Level-3 (100%)	1.50	24782526
Level-4 (120%)	1.80	28829932
Level-5 (150%)	2.25	33851160



Precision

Precision of the method will be calculated by analysing 5 replicate preparations of any one batch sample of test item. The active ingredient (% w/w), mean and % RSD will be calculated. Precision will be reported as the relative standard deviation [(standard deviation/arithmetic mean) x 100]

No of Replicates	Metalaxyl (% w/w)	Prothioconazole (% w/w)	Tebuconazole (% w/w)
1	0.621	1.541	0.299
2	0.620	1.532	0.300
3	0.622	1.551	0.300
4	0.622	1.546	0.301
5	0.624	1.549	0.300
Mean	**0.622**	**1.543**	**0.300**
Stdev	**0.001**	**0.008**	**0.0006**
%RSD	**0.21**	**0.49**	**0.18**

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection and limit of quantitation were evaluated by serial dilution of Metalaxyl, Prothioconazole and tebuconazole from system suitability standard solution. The signal to noise ratio of 3 ± 0.5, will be considered as limit of detection (LOD) and the signal to noise ratio between 5:1 to 10:1, will be considered as limit of quantification (LOQ).

	Metalaxyl (mg/ml)	Prothioconazole (mg/ml)	Tebuconazole (mg/ml)
Limit of Detection	0.000102	0.000101	0.000106
Limit of Quantitation	0.00102	0.00101	0.00106

Accuracy and recovery: Accuracy (% Recovery) of analytical method was determined at four concentration levels by spiking known amount of pure actives in sample i.e., LOQ, 80%, 100% and 120%. The accuracy was calculated as % of recovery

Components	Level	Amount added*(mg/ml)	Amount found*(mg/ml)	% Mean Recovery
Metalaxyl	LOQ	0.00102	0.00101	99.02
	80%	0.48045	0.47813	99.52
	100%	0.60056	0.59625	99.28
	120%	0.72068	0.71352	99.01
Prothioconazole	LOQ	0.00101	0.00100	99.01
	80%	1.20022	1.19035	99.18
	100%	1.50028	1.50032	100.0
	120%	1.80033	1.79067	99.46
Tebuconazole	LOQ	0.00106	0.00104	98.11
	80%	0.24007	0.23738	98.88
	100%	0.30008	0.29862	99.51
	120%	0.36010	0.36020	100.03

Robustness: The robustness of the method is unaffected when small, deliberate changes like, flow (1.0 ± 0.10 ml/min), change, mobile phase composition 0.1% OPA: Acetonitrile (40 ±5: 60 ±5)and column temperature (30°C ± 5°C) were performed. It was found that % purity values were unaffected after these small variations.

Conclusion :

A simple precise, accurate, rapid, less analysis time consuming and economical HPLC method has been developed for quantification of of Metalaxyl, Prothioconazole and tebuconazole in their pesticide formulation. Method validation study showed that the method is specific, linear, accurate, robust, and easily reproducible. This method is also useful for quantification of Metalaxyl, Prothioconazole and tebuconazole in their single or combination formulated products with different strengths and different formulation types. This method can also be

useful for analysis of environmental samples (soil, water), agricultural products for pesticide residue analysis of same actives but required additional extraction procedure. Hence developed method can be adopted to regular quality control analysis of production samples and stability samples, environmental samples. we developed and validated HPLC method—which is simple, rapid, precise, low-cost, and green for the simultaneous determination of different active material and formulated products

References:

ICH," Validation of Analytical Procedures: Text and Methodology," International Conference on Harmonization Guidance Documents, 2005, Q2 (R1).

A. W. Garrison: "Encyclopedia of Analytical Chemistry," ed. by R. A. Myers, John Wiley & Sons, Chichester, pp. 6147–6158, 2000.

Lloyed R. Snyder, Joseph J. Kirkland, John W. Dolan (2010). *Introduction to Modern Liquid Chromatography* (3ed.), John Wiley & Sons.

International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use (1996), Q2B-*Validation of analytical procedures: Methodology*, Geneva.

Lloyed R. Snyder, Joseph J. Kirkland, John W. Dolan (2010). *Introduction to Modern Liquid Chromatography* (3ed.), John Wiley & Sons

SANCO guidelines (2004). *Guidance on Storage Stability Data Requirements for Non-Agricultural Pesticides Products.*

Ragubabu K. Tentu Nageswara Rao, T. B. Patrudu and D. Sreenivasulu. (2012). Determination Of Fungicide Residues in grapes using high-performance liquid chromatography with ultraviolet detection. *Int. J. Cur.Tr.Res. 1*(2), 59-64.

T. Nageshwar Rao, A. Ramesh, T. Parvathamma and G. Suresh. (2012). Development and Validation of HPLC-UV method for simultaneous determination of Strobiurin Fungicide residues in tomato fruit followed by Matrix-Solid Phase Dispersion (MSPD). *Indian J. Sc. Res, 3*(1), 113-118.

Elena Katz, Roy Eksteen, Peter Schoenmakers, Neil Miller (2011). *Handbook of HPLC* (Vol. 78). Chromatographic Science Series.

Analysis of Pesticide Residues. Wako product Information-Chromatography 9 edition. Retrieved from http://www.e-reagent.com.