Stability-Indicating RP-HPLC Method for The Estimation of Propiconazole in its Formulations

G. Rahul[1], B. Ramachandra[2*], N. Venkatasubba Naidu[1],V.K. Somasekhar Srinivas[3,4], Nainaru Tarakaramu[3,4]

[1, 1]Departmentof Chemistry, S.V.University, Tirupati -517502, A.P, India

[2]Department of Chemistry, Humanities and Basic Sciences, Annamacharya Institute of Technology and Sciences, Tirupati-517520, A.P, India

[3] Basic Sciences and Humanities, Mohan Babu University, Sree Sainath Nagar, Tirupati-517102, A.P, India

[4]Basic Sciences and Humanities, Sree Vidyanikethan Engineering College, Sree Sainath Nagar, Tirupati-517102, A.P, India

*Corresponding Author Mail ID: ***brcsvu@gmail.com***

ABSTRACT

In the present study, RP-HPLC Method used to analysis of Propiconazole in pharmaceutical formulations. Composition of mobile phase is water and acetonitrile in the ratio 50:50 (v/v), respectively and produces a strong peak of Propiconazole after 15 minutes of use. Propiconazole was studied an HPLC analysis at a wave length of 210 nm and the flow rate is 1.0 mL/min,correlation coefficient of 0.999 and the linear regression is y = 3410x - 157.7 for the analysis of Propiconazole, the devised methodology was used with a high level of accuracy and precision. Accuracy, precision, resilience, toughness, and specificity of the approach were all verified.

Keywords: Forced Degradation;Propiconazole;HPLC; ICH recommendations; Method Validation.

INTRODUCTION

Propiconazole is an N-substituted triazole. Due to its affinity for the 14-alpha demethylase enzyme and ability to prevent it from demethylation an ergo sterol precursor, sometimes it is referred as a demethylation inhibiting fungicide. It belongs to the class of fungicides that impede ergosterol biosynthesis because without this demethylation step, the ergosterols cannot be absorbed into the fungal cells that are developing and cellular

proliferation is halted. To control the powdery mildew,needle/leaf and stem fungus on hardwoods and conifers as well as on barley, grapes, peanuts, pineapples, sugarcane, barley, wheat, etc.Propiconazole is used as a fungicide. Propiconazole in is now approved for residential usage as a wood preservation treatment, as well as for lawn and decorative purposes.Fungicides are applied to a variety of vegetable, fruits, and ornamental crops to avoid foliar diseases. They are typically used before illnesses develop since they are normally more successful as preventative measures than as therapeutic ones. Fungicides can travel off of fields after application, just like other pesticides can, and contaminate nearby surface water, groundwater, and associated sediments. Spray drift and runoff are two ways that Propiconazole can get into the aquatic environment. In water, Propiconazole is extremely soluble. It seems to undergo a gradual photo transformation and remains stable throughout hydrolysis. Propiconazole has moderate to persistent persistence in aquatic environments. The principal transformation products are two molecules that have been hydroxylated at the diesoline moiety, and biotransformation is a significant pathway of transformation. Propiconazole readily disperses from water to soil or sediment and persists in anaerobic environments. Therefore, when there is soil erosion due to heavy rainfall, Propiconazole may contaminate aquatic environments through off-site runoff. Although it has been found, the frequency of discovery of Propiconazole is minimal. Bioaccumulation is not anticipated to be a big concern for Propiconazole because of its quick depurative action. An efficient normal phase HPLC method for determining and validating the presence of Propiconazole enantiomers in water, soil, and grapes was reported by Youpu Cheng et al. [1] and Mu Wei and co et al. [2] Supercritical fluid chromatography-tandem mass spectrometry wasused determine the Propiconazole by Youpu Cheng et al. [3] to establish an effective and sensitive chiral analytical technique for determining Propiconazole stereoisomer's, by Wang Chunwei et al.[4] HPLC/Ms methoduse for determination of Propiconazole and azoxystrobin residues by Sizhuo Wu et al. [5] LC-Electro-spray ionisation tandem mass spectrometry method was developed for the detection of the fungicides difenoconazole, Propiconazole, and pyraclostrobin in peppers and soil. Two distinct analytical techniques were created and verified by A. Blondel et al. [6] In this present study the author developed a analytical method for the determination of Propiconazole by using the RP-HPLC Method.

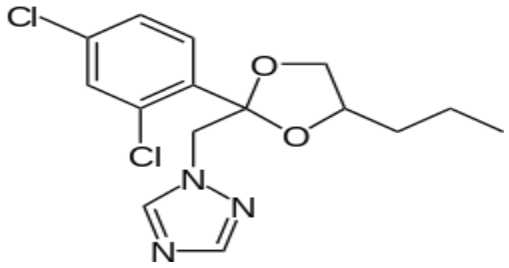


Fig. 1 Chemical structure of Propiconazole

Chemical name: 1-[[2-(2, 4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-yl] methyl]-1, 2, 4-triazole

Empirical formula: $C_{15}H_{17}Cl_2N_3O_2$

Molecular weight: 342.220g/mol^{-1}

INSTRUMENTS / EQUIPMENTS REQUIRED

HPLC instrument with a UV/PDA detector, HPLC analytical column of ECLIPSE XDB-C18 - 250mm x 4.6mm x 5, analytical weighing scale of Mettler Toledo B204S, Millipore Nylon 0.2m, and laboratory accessories are the instruments we used in this approach.

CHEMICALS REQUIRED

Propiconazole working sample, Propiconazole UNGICIDE 250 EC, Methanol- AR, Sodium Hydroxide- AR, Hydrochloric Acid- AR, Acetonitrile, and Millipore Water are the chemicals utilized in this procedure. A UV/VIS detector-equipped HPLC system performs the quantitative analysis.

Mobile Phase : Prepare a 50:50 mixture of water and acetonitrile for theisocratic elution. Combine well. Filter and degas before using with 0.2 Nylon membrane filter paper.

Propiconazole Standard Solution Preparation:

Transfer the Propiconazole working standard that has been precisely weighed (50mg), taken in to a 50ml volumetric flask. To dissolve, sonicate 20 ml of diluent after adding it. Mix after diluting with diluent to volume. Pipette out 1.0ml of Propiconazole sample in to a 10ml volumetric flask, made it in to a mark with diluent and mixed well.

PropiconazoleTest solution Preparation:

Transfer the material to a 50 ml volumetric flask after precisely weighing approximately 200 mg of it. 20 ml of diluent should be added, then dissolved using a sonicate. Mix after diluting with the diluent to volume. Pipette out 1.0ml of Propiconazole sample in to a 10ml volumetric flask,made it in to a mark with diluent and mixed well.

Solution for System Suitability:

Procedure: Follow the steps below to inject five duplicate injections of the system suitability solution onto equal quantities of blank paper (Propiconazole Standard working solution). After injecting the test solution twice, capture the chromatograms. Any peak brought induced by a blank in the test solution must to be disregarded. Determine the system-appropriate solution's percent RSD for five duplicate injections. Examine the chromatogram created by the fifth injection of the system suitability solution to confirm the theoretical plates and tailing factor for the peak. The limitations are as follows:

1) Theoretical plates more than 2000.

2) Tailing factor is less than 2.0.

3) % RSD is less than 2.0%.

VALIDATION PARAMETERS [7]

Throughout the validation investigation, the system suitability parameters were tracked and are documented in the validation report. Below is a summary of the validation data:

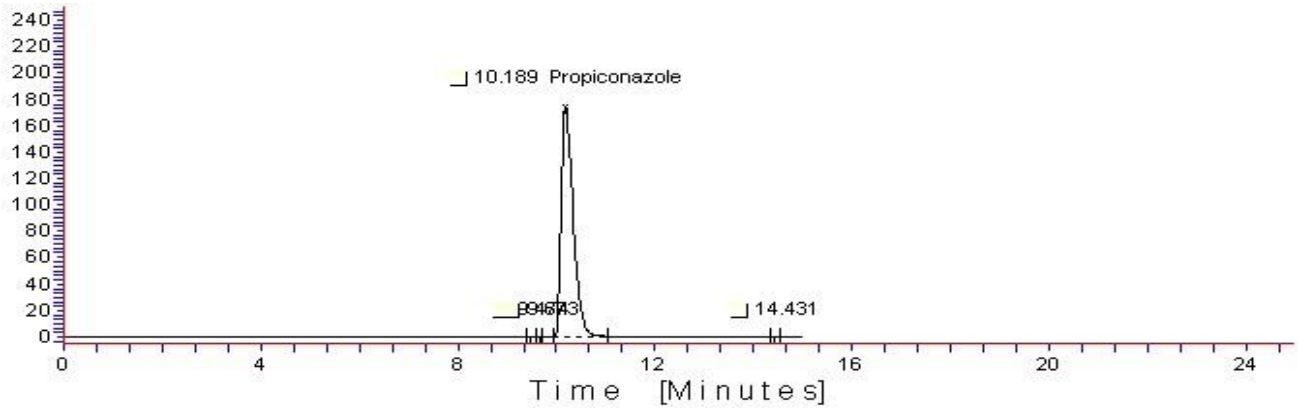
Selectivity: Selectivity was attained by injecting the test solution, the excipient mix, the system suitability solution, and the diluent blank solution. Acceptance criteria:The Propiconazole summit must be clearly separated from all other peaks and from one another. There should be no peak in the excipient blend solution or diluent blank solution at the Propiconazole retention time. The analysis procedure revealed that the system suitability criteria satisfied the previously set acceptance requirements. % RSD was found to be 0.71%. The method's prescribed wavelength was used to process each injection. Diluent blank solution and excipient mix solution containing Propiconazole peak did not cause any interference, according to the results. It's a selective method.

FORCED DEGRADATION

In order to find out what stability indicating characteristics the test method possessed and to look for any degraded compounds, a forced degradation analysis was carried out.5N HCl, 5N NaOH, heat degradation, and UV degradation are used to stress the Propiconazole WS and the sample.Each of the aforementioned solutions has a chromatogram that was captured using chromatography.Deterioration takes place in the following stressful circumstances.

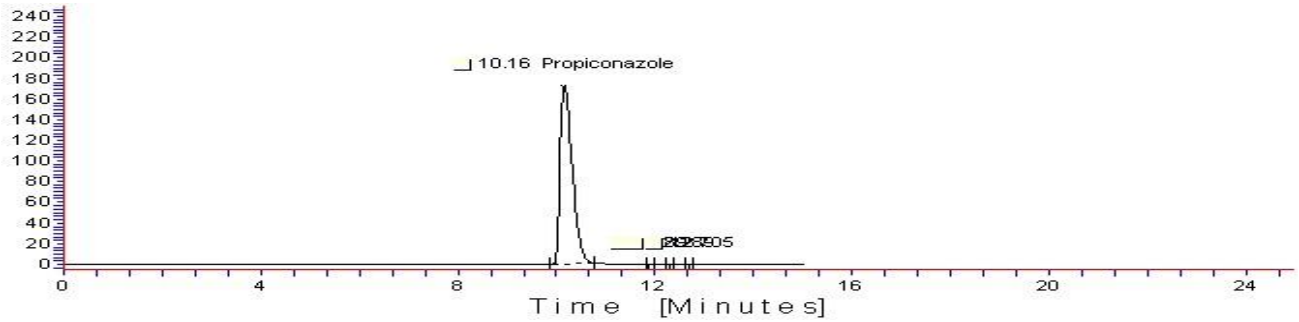
Table. 1Suitability of the System - Forced Degradation study

S.No.	Area of Propiconazole
1	2951.13
2	2949.15
3	2956.43
4	2922.75
5	2968.93
Mean	**2949.68**
SD(±)	**16.91**
(%) RSD	**0.57**



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	9.474	0.7	0.096	0.024	0.055	0.133
2	9.673	0.093	0.017	0.003	0.01	0.05
3	10.189	2969.208	174.919	99.954	99.896	0.283
4	14.431	0.565	0.07	0.019	0.04	0.15
Total		**2970.566**	**175.102**	**100**	**100**	

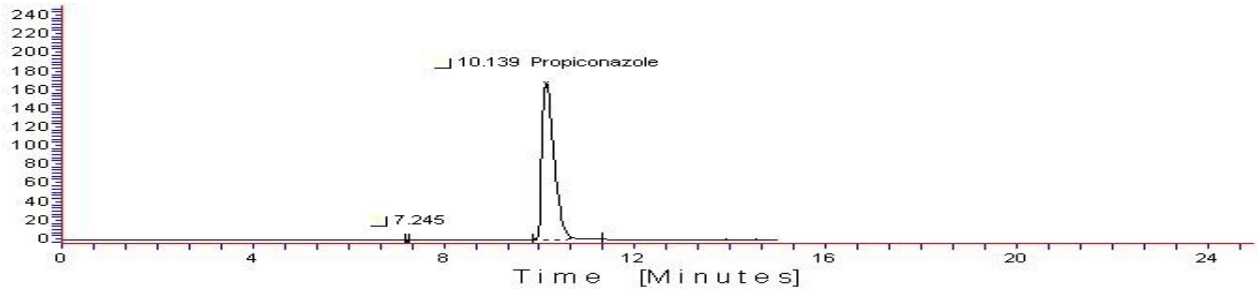
Fig. 2Propiconazoledegradation sample chromatogram in acid



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	10.16	2957.026	172.897	99.953	99.869	0.283
2	11.89	0.523	0.077	0.018	0.045	0.133

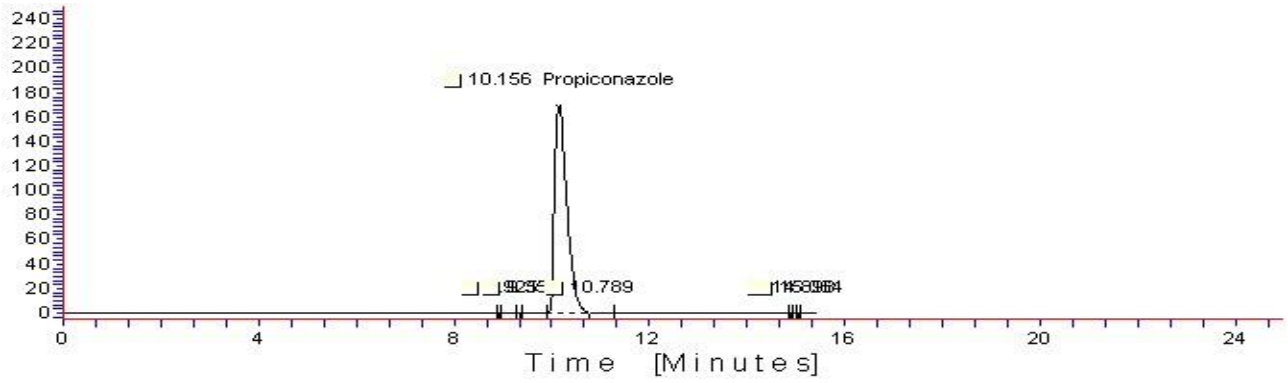
3	12.289	0.386	0.075	0.013	0.043	0.1
4	12.705	0.486	0.075	0.016	0.043	0.116
Total		**2958.421**	**173.124**	**100**	**100**	

Fig. 3 Propiconazoledegradation sample chromatogram in Alkali



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	7.245	0.098	0.041	0.003	0.024	0.05
2	10.14	2931.633	169.369	99.997	99.976	0.283
Total		**2931.731**	**169.41**	**100**	**100**	

Fig. 4Propiconazoledegradation sample chromatogram in Thermal



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	8.925	0.129	0.035	0.004	0.021	0.066
2	9.358	0.173	0.037	0.006	0.022	0.083
3	10.157	2937.981	170.525	99.738	99.386	0.283
4	10.789	7.094	0.863	0.241	0.503	0.116
5	14.898	0.191	0.065	0.006	0.038	0.05
6	15.064	0.134	0.054	0.005	0.031	0.05
Total		**2945.702**	**171.579**	**100**	**100**	

Fig. 5Propiconazoledegradation sample chromatogram in UV

Acceptance Criteria:

There should be sufficient distance between the degradation peaks. Propiconazole should pass the peak purity test. The peaks found on the chromatograms of the degradation preparations are not interfering with one another. The forced deterioration's degradation peaks are clearly distinct from one another. Propiconazole is passing through its purest state. Since the degraded products are clearly distinguished from Propiconazole and from each other's neighboring peaks, the method is particularly exact, selective, and specific for the estimate of the assay of Propiconazole Fungicide 250 EC by HPLC

method.Propiconazole sample degraded in acidic condition 0.046%, basic condition 0.047%, thermal condition 0.003% and UV condition 0.262% respectively.

Linearity

Sample linearity and range:

The five Propiconazole sample solutions in the range of 50% to 150% of the predicted assay preparation concentration were used for linearity study. The solutions for linearity study were injected in accordance with the procedure. The correlation coefficient was obtained by generating a graph b/w against to the concentration vs peak area, correlation coefficient is 0.999. The results are shown in the table -2.

Table. 2 Results of sample linearity

Linearity	Sample Concentration (in %)	Sample Concentration(inppm)	Peak Area	Correlation Coefficient
Sample -1	50	50	1519.38	
Sample -2	75	75	2462.94	
Sample -3	100	100	3229.87	**0.999**
Sample- 4	125	125	4072.04	
Sample -5	150	150	4977.45	

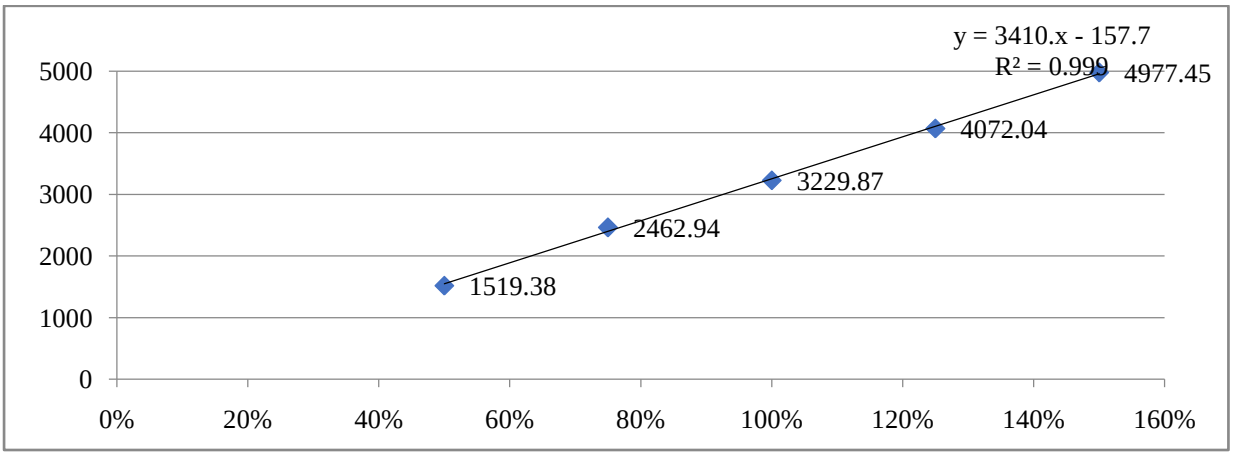
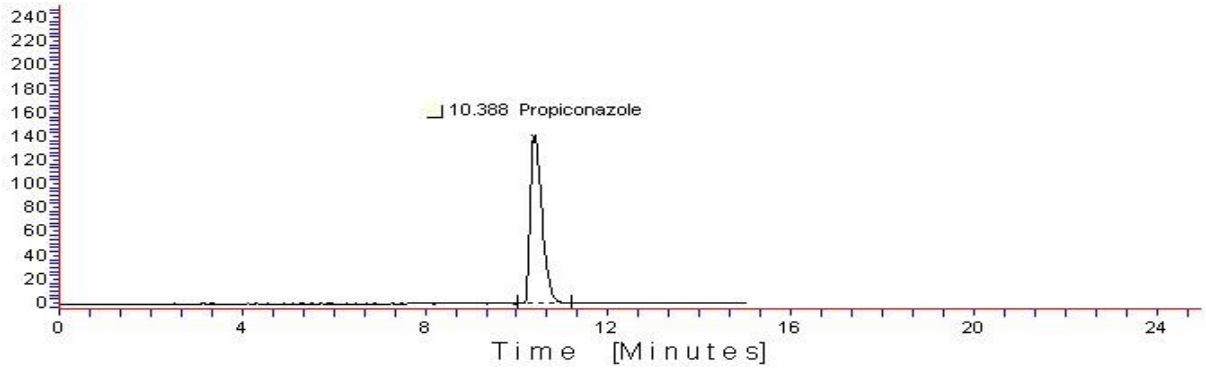


Fig.6Propiconazole sample Linearity graph



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	10.389	2462.943	141.522	100	100	0.283
Total		**2462.943**	**141.522**	**100**	**100**	

Fig. 7Propiconazole sampleChromatogram

Precision:

System Precision:

Procedure:

By studying the system precision and the chromatograms were checked for conformity with the system suitability requirements, by injecting 10 samples of the system suitability solution. The Percentage of RSD is must be lessthan 2.0%. The analysis process revealed that the system suitability criteria satisfied the previously defined acceptance standards. The results are given in table -3.

Table. 3Results of System precision

S. No.	Area of Propiconazole
1	2962.16
2	2964.05
3	2957.92
4	2955.71
5	2951.32
6	2950.33
7	2956.48
8	2955.00
9	2941.44
10	2943.09
Mean	**2953.75**
SD (±)	**7.38**
(%) RSD	**0.25**

Method Precision:

Procedure:

The method precisions of Propiconazole in the Amistar Fungicide 250 EC wereprepared in accordance with the analytical procedure. The % RSD of method precision

of sample solutions is less than 2.0%. The analysis process revealed that the predetermined acceptance conditions were met by the system appropriateness criterion; results are given in tablae-4.

Table. 4 Results of Method precision

Test Solution	% Assay of Propiconazole
1	99.87
2	98.99
3	99.26
4	99.35
5	100.06
6	100.94
Mean	**99.75**
SD (±)	**0.71**
(%) RSD	**0.71**

Study of Intermediate Precision:

Procedure:

According to the aforementioned analytical process, six sample test solutions of the Propiconazole in Amistar Fungicide 250 EC were created on various days. The samples examined by a different analyst using different HPLC equipment and a different HPLC column of the same brand but with a different serial number. Six samples from the method precision and six samples from the intermediate precision were used to determine the %RSD of the assay findings for the 12 test solutions. The percentage RSD of the findings from the twelve test solutions must be less than 2.0% in order to meet the acceptance criteria. The analytical method found that the system suitability criteria satisfied the previously stated acceptance standards. Table 5 shows the assay findings obtained from the six test solutions.

Table.5Results of Intermediate precision

Test Solution	% Assay of Propiconazole
1	99.16
2	98.63
3	99.37
4	98.74
5	99.78
6	98.80
Mean	**99.08**
SD (±)	**0.44**
(%) RSD	**0.45**

Robustness:

In line with the analytical technique, prepare two test solutions using the same lot of Propiconazole in Amistar Fungicide 250 EC. The following chromatographic conditions should be injected together with this solution, the system suitability solution, the diluent blank solution, and other solutions: The same batch of Amistar Fungicide 250 EC Propiconazole was tested at different flow ratesie is 0.8mL/minute and 1.2mL/minute and the %RSD was 0.35 and 1.03, wavelengths 208nm & 212nm and the %RSD was 0.39 and 0.13, column lots we used same column and different column % RSD was 0.09 and 0.01 and mobile phase compositions are acetonitrile and water in the ratio 480:520v/v & 520:480v/v and the %RSD was found to be 1.03 &0.01 respectively. It was concluded that the system was appropriate at all times since the %RSD between outcomes generated in various scenarios and the method's average accuracy result was less than 2.0%. The analytical Method meets the previously defined acceptance requirements from the protocol's robustness evaluation. Because of this, the Method is accurate.

CONCLUSION AND SUMMARY

As stated in the Summary & Conclusion above, the analytical methodof the Propiconazole test in Amistar in 250 EC Fungicide by HPLC method is selective, specific, linear, accurate, and robust. This is supported by the validation results from this study. At room temperature, this solution is stable for up to 48 hours. Therefore, it can be concluded that the analytical approach is verified and that it may be used for both regular analysis and stability research.

ACKNOWLEDGEMENTS

The author is thankful to the Department of Chemistry at Sri Venkateswara University in Tirupati, India, and Analog Labs in Hyderabad, India, for letting him use their labs.

REFERENCES

1. Cheng, Y., Dong, F., Liu, X., Xu, J., Li, J., Chen, X.,& Zheng, Y. (2013). Stereoselective separation and determination of the triazole fungicide propiconazole in water, soil and grape by normal phase HPLC. Analytical Methods, 5(3), 755-761.
2. Wei, M., Feng, L., Zuoyang, S., Guang, W., &Lianlai, Y. (2005). Study on the Quantitative Analysis of Tebuconazole, Propiconazole and Triadimefon by GC and HPLC [J]. Pesticide Science and Administration, 7., 1-3
3. Cheng, Y., Zheng, Y., Dong, F., Li, J., Zhang, Y., Sun, S.,& Zhang, W. (2017). Stereoselective analysis and dissipation of propiconazole in wheat, grapes, and soil by

supercritical fluid chromatography–tandem mass spectrometry. Journal of agricultural and food chemistry, 65(1), 234-243.

4. Wang, C., Wang, Y., Wang, R., Yan, J., Lv, Y., Li, A., & Gao, J. (2017). Dissipation kinetics, residues and risk assessment of propiconazole and azoxystrobin in ginseng and soil. International Journal of Environmental Analytical Chemistry, 97(1), 1-13.
5. Wu, S., Zhang, H., Zheng, K., Meng, B., Wang, F., Cui, Y., ... & Hu, D. (2018). Simultaneous determination and method validation of difenoconazole, propiconazole and pyraclostrobin in pepper and soil by LC–MS/MS in field trial samples from three provinces, China. Biomedical Chromatography, 32(2), e4052.
6. Blondel, A., Krings, B., Ducat, N., & Pigeon, O. (2018). Validation of an analytical method for 1, 2, 4-triazole in soil using liquid chromatography coupled to electrospray tandem mass spectrometry and monitoring of propiconazole degradation in a batch study. Journal of Chromatography A, 1562, 123-127.
7. ICH Guideline number: Q2A & Q2B of CPMP / ICH / 281 / 95.