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Method validation and assessment of dislodgeable foliar residue of chlorantraniliprole on maize

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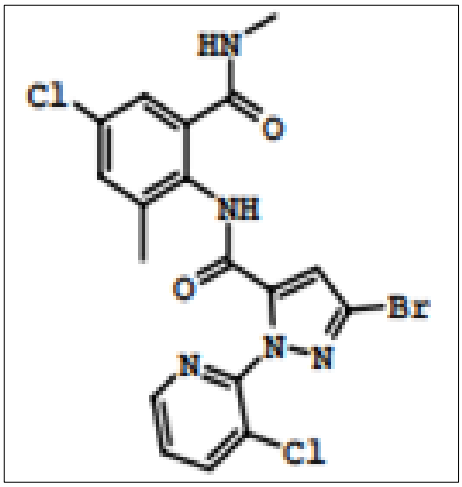
Abstract
A simple, reliable and sensitive reverse-phase high-performance liquid chromatography method with photodiode array detector was developed and validated for analysis of the dislodgeable foliar residues of chlorantraniliprole on maize leaf. Chromatographic conditions for reversed - phase HPLC with photodiode array detection were as follows: column, C18 (Agilent) column, 25 length x 4.6 cm, 5 µm; column temperature, 40 °C; mobile phase, a 70:30 (v/v) acetonitrile: water; flow rate 1 mL/min. The detection wavelength was UV 254 nm. A good linear relationship R^2 = 0.999 was obtained for chlorantraniliprole from 0.05 to 3.2 µg/ml. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.007 ng/cm^2 and 0.023 ng/cm^2 respectively. Recoveries were 94.53 ± 0.70% and precision were 0.74 to 2.58%. The proposed method was successfully applied to analysis of the dislodgeable foliar residues on maize leaf.

Keywords: maize, method validation, dislodgeable foliar residues, chlorantraniliprole

1. Introduction
Maize (*Zea mays*) known as queen of cerealsis the third most important cereal in India next to rice and wheat. In India, Maize is cultivated on 9.2 million hectares with an annual production of 27.82 million metric tonnes (FAO, 2018) [1]. Chlorantraniliprole (3-bromo-1-(3-chloropyridin-2-yl) -1H- pyrazole-5- carboxylic acid) is the first of the anthranilic diamide insecticides, a ryanodine receptor activator belonging to a new class of selective insecticides with a novel mode of action used to control fall armyworm in maize (USEPA, 2008) [2]. The residues present on both the surface of leaves and the stem that may be washed off from the leaf surface is known as dislodgeable foliar residue. Which residing on plant surfaces can be transferred to worker skin and clothing and result in dermal exposure of workers to toxic materials (Choi *et al*., 2013) [3]. Short and intermediate term dermal exposure of Chlorantraniliprole do not pose a systemic risk (EPA, 2008) [4]. According WHO recommended classification of pesticides by hazard, chlorantraniliprole has been classified under yellow denotes that the pesticide listed does not pose a risk at commonly applied application rates (Prasanna *et al*. 2017) [5]. Validating method mainly done according to definite protocol examination parameters such as linearity, accuracy (recovery), precision (repeatability and reproducibility), detection and quantitation limit (ICH, 2005) [6].
Dislodgable Foliar Residue (DFR) extraction procedure which was initially developed by Gunther and co-workers (Gunther *et al*. 1974; Westlake *et al*. 1973) [7, 18]. Extraction of foliar dislodgeable residues with a water - detergent solution and then partitioning the residues into dichloromethane is the commonly followed method developed by (Iwata *et al*. 1977) [8]. There are two sampling method for DFR were leaf punch samples and whole leaf sample. The result can be expressed in both ppm and μg/cm^2. In this study the dislodgable foliar residue sample were processed according to method suggested by (Iwata *et al*. 1977) [8] and (Kuttalam, 1990) [9] with some modification for collecting and removing dislodgeable residue.



Chlorantraniliprole

2. Materials and Methods

2.1 Reagent and standards

The analytical standard for chlorantraniliprole was purchased from Sigma Aldrich, Bangalore and were of > 98% analytical purity. HPLC grade acetonitrile were purchased from Merck India Ltd., Mumbai. MilliQ ultra (Q3 Merck Millipore unit) purified water was used in this experiment. Laboratory grade solvents like dichloromethane, hexane, anhydrous sodium sulphate and sodium chloride also purchased from Merck India Ltd. Mumbai.

2.2 Preparation of standard solution

Standard stock solution of chlorantraniliprole (400 mg/L) was prepared in HPLC grade acetonitrile by dissolving 10.18 mg of the analytical standard into a calibrated Class A volumetric flask (25 mL). The required standard solutions for plotting of calibration curve (0.05 to 3.2 ppm) were prepared from stock solution by serial dilution with acetonitrile. All standard were stored in -20 ˚C until further use.

2.3 Extraction and clean-up

Maize leaves disc were used as substrates for standardization of the methodology for estimation of chlorantraniliprole dislodgeable residues. Leaf disc were collected according to guidliness given by (Edmiston, 2002) [10]. Fifty leaf disc of 3 cm diameter were collected from maize plant with a leaf punching machine (Figure 1) in a wide mouthed bottle containing 150 ml double distilled water with 16 drops of teepol. The samples were quaked well in a reciprocal shaker at 2000 rpm for 20 min. Extract were collected in 1000 ml beaker. Residues in the leaf samples were extracted with additional 200 ml distilled water thrice. 150 g of sodium chloride was added with the extract and shacked well. The pooled aqueous extracts were taken in a 1000 ml separating funnel with 75 ml of dichloromethane and shudder well for 1 min. Lower organic layer was drained through funnel containing 10g anhydrous sodium sulphate into conical flask. Process repeated with 50 ml DCM twice. Final extract was concentrated to near dryness in rotary vacuum evaporator at 35 °C and repeated 3 times with 10 ml hexane. Finally, 5 ml acetonitrile was added and taken to test tube and dried using turbo evaporator. Residue was collected in 1.5 ml vial using 1 ml of acetonitrile in the test tube by filtering through syringe filter. Vial kept in the auto sampler in the UHPLC for Injection.

Fig 1: Collecting leaf disc with leaf punching machine

2.4 Equipment and chromatographic Conditions

The Ultra High Performance Liquid Chromatograph (UHPLC) system (Shimadzu LC 2030 C) equipped with an auto injector, a system controller Lab Solution, a pump, a column oven, and a photodiode array detector was used for the study. Acetonitrile: water (70: 30, v/v) was used as mobile phase with the flow rate of 1 ml/min. The mobile phase was filtered through 0.45µ pore size membrane filter and the dissolved gases were removed ultrasonically. Ten µL of the samples were injected. Reverse phase - C18 (Agilent) column (250 mm length x 4.6 mm id x 5 µ particle size) was used for separation of residues. The column oven temperature was maintained at 40 °C. The detection was carried out at wavelength 254 nm. The run time for the analysis was 10 min.

2.5 Method validation

The developed method was validated as per as per SANTE guidelines (SANTE, 2017) [11] for selectivity, specificity, linearity, limit of detectionand quantification, recovery, accuracy and presision, matrix effect for validation of analytical procedures.

The selectivity of the method was determined by comparing the HPLC chromatograms of six blank samples to six spiked samples. There were no peaks to be found in the reagent blank at the retention times of chlorantraniliprole. The developed analytical method needs to be specific and conformed by obtaining positive results from maize leaf disc. To study linearity, detector response for chlorantraniliprole was studied by injecting standard solutions in the range of 0.05 to 3.2 µg/ml in UHPLC with 3 injections per concentration. The peak areas versus concentrations data were evaluated by linear regression analysis and the respective equation was developed at 95% confidence interval. The sensitivity of the method was assessed by arriving the limit of detection (LOD) and limit of quantification (LOQ) by spiking the chlorantraniliprole with selected matrices at the lowest concentration level meeting the analytical method requirements. The LOD and LOQ were determined by seeing the signal-to-noise ratio of three and ten, respectively with regarding the background noise achieved from the blank matrices

The recovery test was performed on control (blank) samples leaf disc by spiking the standard solution of chlorantraniliprole at three different concentrations (0.05, 0.25 and 0.5 µg/g) and replicated three times along with control separately. The precision of the present method was performed in terms of repeatability (Relative Standard Deviation) for each spiked levels of 0.05, 0.25 and 0.5 µg/g of the selected matrices. By comparing the responses obtained

from solvent standards and matrix matched standards, the matrix effect (ME) was determined. The ME was assessed with the help of following formula

$$ME\% = \frac{(\text{Peak area of the matrix} - \text{Peak area of solvent})}{\text{Peak area of solvent}} * 100$$

3. Results and Discussion

The results of the method validation study found that, the method was found to be specific, with no mobile phase interfering on the resultant peak. For both the standard and sample solutions, the peak purity was 0.999 and 0.996 percentiles, respectively. Selectivity was assessed by comparing chromatograms of six different types of blank leaf disc with the corresponding spiked sample with chlorantraniliprole at 0.5 µg/g. No significant interference from endogenous substances with chlorantraniliprole was detected. Typical retention times for chlorantraniliprole were 4.92 min, respectively. The short retention time of 4.92 minutes ensured quick analysis of sample. Thus, interference from leaf disc matrix was negligible in this method.

The detector response was linear for the chlorantraniliprole concentration range 0.05 – 3.2 µg/ml (R^2 = 0.999, 95% confidence interval) and linear regression equation y = 20828.93x -397.00 for standards (Figure 2&3). The LOD and LOQ for maize leaf disc was 0.007 ng/cm^2 and 0.023 ng/cm^2, respectively (Table 1). The mean recoveries of chlorantraniliprole from leaf disc were 94.53 ± 0.70% respectively (Table 1). According to SANTE guidelines the recovery fall within the range of 80 – 120% for recovery hence the method is accepted. The precision of the analytical method in terms of RSD was in the range of 0.74 to 2.58%. The matrix effect account for 2.41 to 3.65% of the spiked concentration of chlorantraniliprole in the maize leaf disc. Initial attempts to separate compounds by using methanol in the mobile phase provided poor resolution. But, when acetonitrile was put into the mobile phase, the separation of analytes was optimised, with appropriate resolution. The sharper the peak, the better is the column efficiency.

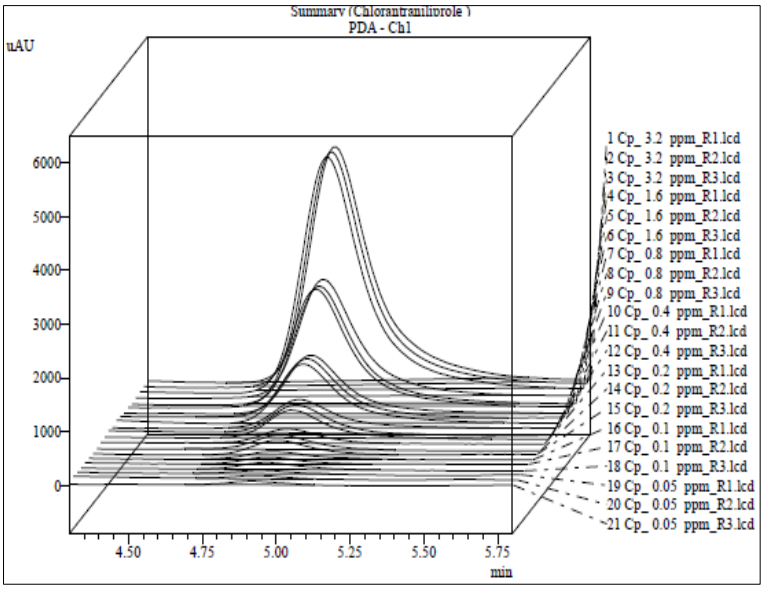


Fig 2: Linearity of chlorantraniliprole in UHPLC

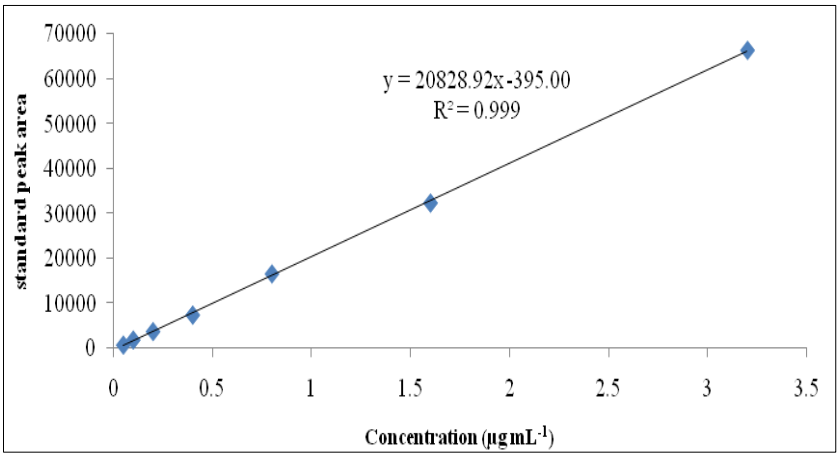


Fig 3: Calibration curve of chlorantraniliprole in UHPLC-PDA

The practical applicability of the newly developed method was assessed by the analysis the dislodgeable foliar residues of chlorantraniliprole (40 g ai/ha) on maize under high volume spraying system. On the day after application, the dislodgeable residues detected on the maize leaves were 5.43 ng/cm^2 (Table 2). A day after application, a rapid decrease of about 66.2% of the initial deposit was detected and disappeared within 5 days. The rate of disappearance of dislodgeable residues of chlorantraniliprole as indicated by the minimum half-life of 0.89 days. The above results are in

accordance with the results (Evaristo, 2002) [12] who reported Half-life values for dislodgeable methamidophos residues as 0.90 days.

Table 1: Dislodgeable foliage residue of chlorantraniliprole in maize in High Volume Sprayer

Days after spraying	Residue level in ng/cm^2			Mean
	R1	R2	R3	
0	5.43	5.20	5.33	5.32
1	1.83	1.76	1.57	1.72
3	0.31	0.27	0.29	0.29
5	BDL	BDL	BDL	BDL

The parameter that affect the persistence of dislodgeable residue were weather conditions, such as rain, relative humidity and temperature (Nigg & Stamper, 1982) [13] and crop type, pesticide formulation type and sprayer type (Kasiotis, 2017) [14], The above values are lower than the values reported by Ganesan (2004) [15], who reported dislodgable residues of endosulfan in rice with 28.58 ng/cm2 of initial deposit under high volume spraying system. According to (Giles, 1991) [16] Initial dislodgeable foliar residue from the reduced-volume application was higher (7.03 vs 5.33 µg/cm2) than from a conventional, high-volume (1870 L/ha) application.

The result of the method validation shows that the procedure for determination dislodgeable foliage residues of chlorantraniliprole in maize is much simpler when compare to previous described methods. No analytical problems were encountered in processing of sample of dislodgeable residue in maize leaf. The present analytical method for chlorantraniliprole offers relatively convenient, sensitive and relaiable for measurement of foliar dislodgeable residue.

4. Conclusion

The UHPLC method for analysis of the chlorantraniliprole was successfully developed and validated. It was rapid, reliable and sensitive. All validated parameters of the method were in accordance with SANTE guidelines. The method was successfully applied to analysis dislodgeable foliar residues of chlorantraniliprole on maize leaf.

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Table 2: Recovery of chlorantraniliprole in leaf disc

		R1	R2	R3	Mean*(%) ± SD	RSD (%)		
Leaf disc (µg/cm^2)	0.05	94.13	92.76	93.69	93.53 ± 0.70	0.74	0.007	0.023
	0.25	92.86	97.55	93.83	94.75 ± 2.47	2.58		
	0.50	93.80	94.72	91.56	93.36 ± 1.62	1.72		

6. References

1. FAO. World Food and Agriculture – Statistical Pocket Book 2018. Rome. Licence: CC BY-NC-SA 3.0 IGO 2018, 1-254.
2. USEPA. Pesticide fact sheet: chlorantraniliprole. U.S. Environmental Protection Agency Washington, DC 2008.
3. Choi H, Byoun JY, Kim JH. Determination of reentry interval for cucumber harvesters in greenhouse after application of insecticide methidathion. Journal of the Korean Society for Applied Biological Chemistry 2013;56(4):465-467.
4. EPA. Pesticides Fact Sheet. Chlorantraniliprole 2008;7505P:1-34.
5. Prasanna BM, Huesing JE, Eddy R, Peschke VM. Fall armyworm in Africa: A guide for integrated pest management. CIMMYT 2018, 1-120.
6. ICH. Validation of Analytical Procedures: Text and Methodology Q2(R1). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. 2005. [Visited on 11 Oct, 2021].
7. Gunther FA, Barkley JH, Westlake WE. Worker environment research. II. Sampling and processing techniques for determining dislodgable pesticide residues on leaf surfaces. Bulletin of Environmental Contamination and Toxicology 1974;12(6):641-644.
8. Iwata Y, Spear RC, Knaak JB, Foster RJ. Worker reentry into pesticide-treated crops. I. Procedure for the determination of dislodgable pesticide residues on foliage. Bulletin of Environmental Contamination and Toxicology 1977;18(6):649-655.
9. Kuttalam S. Occupational Exposure of Field Workers to the Insecticides applied in Cotton Ecosystem. Phd diss. Tamil Nadu Agricultural University, Coimbatore 1990.
10. Edmiston S. Guidance for determination of dislodgeable foliar residue. California Environmental Protection Agency, Department of Pesticide Regulation, Worker Health & Safety Branch 2002.
11. SANTE.2017.<http://ec.europa.eu/food/files/plant/docs/pesticidesmrlguidlineswrkdoc.pdf>.[Weblink: [Visited on 11 oct, 2021].
12. Evaristo A, Baptista GCD. Dislodgeable residues of methamidophos in staked tomatoes. Scientia Agricola 2002;59:469-473.
13. Nigg HN, Stamper JH. Regional considerations in worker reentry. In: Plimmer, j.r. (ed.) Pesticide residues and exposure. Washington: American Chemical Society (ACS Symposium Series, 182) 1982, 69-73.
14. Kasiotis KM, Tsakirakis AN, Glass CR, Charistou AN, Anastassiadou P *et al*. Assessment of field re-entry exposure to pesticides: A dislodgeable foliar residue study. Science of the Total Environment 2017;596:178-186.
15. Ganesan K. Assessment of insecticide exposure in rice eco-system. Phd diss. Tamil Nadu Agricultural University Coimbatore 2004.
16. Giles D, Blewett T. Effects of Conventional and Reduced-Volume, Charged-Spray Application Techniques on Dislodgeable Foliar Residue of Captan on Strawberries. Journal of Agricultural and Food Chemistry 1991;39(9):1646-1651.
17. Gunther FA, Westlake WE, Barkley JH, Winterlin W, Langbehn L. Establishing dislodgeable pesticide residues on leaf surfaces. Bulletin of Environmental Contamination and Toxicology 1973;9(4):243-249.