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Method validation and harvest time residues of chlorantraniliprole 18. 5 SC and cyantraniliprole 10. 26 OD in potato using UHPLC

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Abstract
Field trial was conducted to study the harvest time residues of chlorantraniliprole and cyantraniliprole on potato variety Kufri Jyoti at farmers' holdings in Kukkal village, Kotagiri, (11. 46°N 76. 88°E and 1,847 MSL) Nilgiris District, Tamil Nadu, during the summer season of 2021. At the time of harvest, samples are collected. The tubers were sliced into small pieces in the lab, and a sub sample of around 500 g was taken and homogenised using a high-speed mixer grinder. In UHPLC, the parameters of specificity, linearity, LOD, LOQ, recovery, and repeatability were used to validate the analytical technique. A good linearity was obtained with a correlation coefficient (R^2) of 0. 999 for both chlorantraniliprole and cyantraniliprole. On potato, the LOD and LOQ values for chlorantraniliprole were 0. 021 and 0. 070 µg g^{-1}, respectively, while cyantraniliprole was 0. 003 and 0. 011 µg g^{-1}. Chlorantraniliprole 18. 5 SC @ 30 g a. i. ha^{-1} and cyantraniliprole 10. 26 OD @ 75 g a. i. ha^{-1} residues were found to be below detectable levels in potato tubers collected from the treatment plots at harvest time.

Keywords: Potato, residue, UHPLC, method validation, cyantraniliprole and chlorantraniliprole

1. Introduction
The potato (*Solanum tuberosum L.*; Solanaceae) is one of the world's four major food crops. The potato is a comparatively modern world tuber crop that makes a significant contribution to food and nutritional security, as well as hunger and poverty prevention, particularly in the developing nations whose population demands are rising. A variety of insects can harm potato crops, either directly by feeding on tubers and inflicting harvest impairment, or indirectly by feeding on leaves and stems and transmitting diseases. Leaf miners of the genus *Liriomyza*, which belongs to the family Agromyzidae and the order Diptera, are a serious pests of most vegetables, ornamentals, and weeds all over the world. Given the severity of the damage caused by *Liriomyza* leafminers, it is vital that investigations be carried in potato crops to prevent the pest from spreading and becoming a major pest. Anthranilic diamide are a new class of insecticides that developed from research on an emerging class of insecticidal phthalic diamides with outstanding insecticidal activity against such a spectrum of Lepidoptera. They function by releasing intracellular Ca^{2+} reserves, which would be mediated by the ryanodine receptor [1]. Chlorantraniliprole, [3-bromo-N-[4-chloro-2-methyl-6- [(methyl amino) carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1Hpyrazole- 5-carboxamide], is the first member of the Anthranilic diamide class of chemistry. It has also been reported to be effective against the orders Coleoptera, Diptera, and Hemiptera, in addition to Lepidoptera [2]. When compared to the speed of action of fast-acting carbamates and pyrethroids, the effect of chlorantraniliprole against target pest species is significantly greater than that of most recently developed insecticides in terms of duration for feeding cessation and reduction in feeding damage. The toxicological and ecotoxicological profiles of chlorantraniliprole are both satisfactory. It is effective against insect populations that have developed resistance to other pesticide groups since it belongs to a new chemical class with a novel method of action. Chlorantraniliprole has been categorised as a low-risk pesticide by the Environmental Protection Agency [3]. As a result, chlorantraniliprole is indeed an intriguing new tool for integrated pest management programmes [4]. Cyantraniliprole is a carboxamide generated from chlorantraniliprole with a cyanogroup substituting the chlorine atom connected to the phenyl ring. Cyantraniliprole, (3-bromo-N-[4-cyano-2-methyl-6-[(methylamino)-hydroxy]phenyl]-1-(3-chloro-pyridine-2-yl)-1-H-pyridine-5-formamide, is the second member of the anthranilic diamide class used for the management of lepidopteran pests as well as sucking pests.

Exploration of cyano-substituted anthranilic diamides resulted in the identification of cyantraniliprole, a second compound with outstanding cross-spectrum activity against a wide range of pests from several insect orders [5]. As a result, the current investigation was conducted to determine the chlorantraniliprole and cyantraniliprole residue in potato during harvest time.

2. Materials and Methods

2.1. Chemical and Reagent

The reference standard for chlorantraniliprole (99. 0% purity) and cyantraniliprole (99. 6% purity) was purchased were purchased from M/s. Sigma Aldrich, Bangalore, India. Chlorantraniliprole and cyantraniliprole primary stock solutions of 400 µg ml^{-1} were obtained by dissolving 25. 03, 25. 5, 25. 25, 25. 10, and 25. 06 mg of each in 25 ml of acetonitrile in a volumetric flask. The flasks were labelled and stored at - 20°C in a deep freezer. By diluting appropriate proportions of each pesticide standard solution with acetonitrile, intermediate stock solutions of 100 µg ml^{-1} and 10 µg ml^{-1} were obtained from the primary stock solution. Individual pesticide working standard solutions were prepared by diluting intermediate stock solution to the required volume. The retention time, recovery study, linearity, LOD, LOQ, and quantitative determination of residues in samples were all determined using these working standards. All stocks and working standard solutions were kept at -20°C in the deep freezer until needed. Acetonitrile (CH_3CN) of HPLC grade, sodium chloride (NaCl) and anhydrous magnesium sulphate ($MgSO_4$) of analytical grade were purchased from Merck India Ltd., Mumbai, India. For activation, NaCl and $MgSO_4$ were heated to 650 °C for 4 hours and maintained in a desiccator until needed. Graphitized Carbon Black (GCB) and Primary Secondary Amine (PSA) (Bondesil 40 µm) were purchased from M/s. Agilent technologies, USA. Type 1 water (or HPLC grade water) was harvested from Millipore water purification system.

2.2. Instrument parameters

Ultra High Performance Liquid Chromatography (UHPLC) (Shimadzu, Prominence i series 2030) with auto sampler and Photo Diode Array (PDA) Detector (SPD-M20A) was used to determine the residues of chlorantraniliprole and cyantraniliprole. Chromatographic separation was performed in a column oven at 40°C using a reverse phase (C18 - Agilent) column of 250 mm length, 4. 6 mm id, and 5µ particle size. For separation, an isocratic flow rate of 0. 8 mL min-1 was used with a mobile phase of CH_3CN and H_2O (70:30). The injection volume was 20 µL, with a total run time of 10 minutes. Chlorantraniliprole and cyantraniliprole residues were determined by comparing the peak height / peak area of standards to that of unknown or spiked samples run under identical operating conditions.

2.3. Method validation and measurement uncertainty

Specificity, linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), recovery, repeatability, and ruggedness, as well as measurement uncertainty, were all used to validate the analytical method [6]. By injecting standard solution six times at one concentration (0. 05 µg mL^{-1}), the specificity of the chlorantraniliprole and cyantraniliprole was determined. The linearity test consisted of injecting six different concentrations of chlorantraniliprole and cyantraniliprole, ranging from 0. 01 to 0. 8 µg mL^{-1}, with three replications. Linear regression model was followed to compute LOD and LOQ. The instrument response y is linearly proportional to the standard concentration x for a concentration range of 0. 01 to 0. 8 g mL^{-1} with three replications and expressed in the model y = a+bx for a linear calibration curve. As a result, LOD and LOQ are calculated as LOD = 3Sa /b and LOQ = 10Sa /b, respectively, where Sa is the response's standard deviation and b is the calibration curve's slope. The validity of the current method was tested using recovery studies. For recovery experiments, homogenised untreated potato samples (10g) were spiked with three concentrations of standard solution: 0. 05, 0. 25, and 0. 5 µg g^{-1} with chlorantraniliprole replicated six times and cyantraniliprole replicated three times with an untreated control. The percent recovery was calculated by comparing the peak area of the spiking standards. The control samples were analysed, and the results revealed that the blank sample had no effect on the target molecule. By comparing the peak area of the spiking standards, the % recovery was computed. The sample was spiked at 0. 01 µg g^{-1} level and replicated six times to determine repeatability, reproducibility, and ruggedness. Ellison and Williams (2012) and Magnusson and Örnemark (2012) provided procedures for calculating measurement uncertainty [7, 8].

2.4. Field trial and sampling

During the summer of 2021, a field trial was done to investigate the harvest time residues of chlorantraniliprole and cyantraniliprole on the potato variety Kufri Jyoti at farmers' holdings in Kukkal village, Kotagiri (11. 46°N 76. 88°E and 1,847 MSL) Nilgiris District, Tamil Nadu, India. The experiment was done in a Randomized Block Design (RBD) on a potato variety, Kufri Jyoti, with a plot size of 25 m^2 and each treatment was replicated three times. The field chosen for the experiment had never been exposed to chlorantraniliprole or cyantraniliprole before. At the time of harvest, samples are collected. About 2 kg of potato tubers were randomly harvested from each plot and their residue was analysed. The tubers were sliced into small pieces in the lab, and a sub sample of around 500 g was taken and homogenised using a high-speed mixer grinder. Homogenized samples were stored in wide-mouth glass vials at -20 °C until they were needed.

2.5. Extraction and clean up

The homogenized samples were processed by adopting modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method [9, 10]. After adding 20 ml of acetonitrile, a representative sample of 10 g was transferred into a 50 ml centrifuge tube and mixed for one minute with a vortexer. Following that, four grams of anhydrous magnesium sulphate and one gram of sodium chloride reagents were added, vortexer and agitated again, and the mixture centrifuged at 6000 rpm for ten minutes. After centrifugation, a 6 mL aliquot of the supernatant was transferred into a prefilled 15 mL centrifuge tube containing 100 mg PSA, 600 mg anhydrous Magnesium sulphate ($MgSO_4$), and 10 mg graphitised carbon black (GCB). The mixture was vortexed for one minute before being centrifuged at 3000 rpm for ten minutes. The upper organic layer (4 mL) was placed into a turbovap tube and concentrated to dryness in a turbovap LV at 40 C under a moderate stream of nitrogen. The final volume was reconstituted to one millilitre with acetonitrile and transferred to a 1. 5 mL glass auto sampler vial for analysis using Ultra

High Performance Chromatography (UHPLC) to determine pesticide residues.

2.6. Quantification of pesticide residues

The final quantification was worked out using the following formula with the parameters from chromatogram as

$$\frac{As \times Cstd \times S1 \times Vs}{Astd \times Ws \times Asj}$$

Where

As - Peak area of the sample; Cstd – Concentration of the standard in (µg ml^{-1});

S1 – injected volume of standard (µl); Vs –volume of the sample (final extract in mL);

Astd - Peak area of the standard; Ws - Weight of the sample in g; Asj- Aliquot of the sample injected in µl.

3. Result and Discussion

Method validation is a set of tests that assess any assumptions that the analytical method is based on, as well as establish and document the method's performance characteristics, demonstrating whether the method is suitable for a certain analytical purpose [11]. The method was validated using the parameters specificity, linearity, LOD, LOQ, recovery, and repeatability. The current method was developed by considering the findings of several preliminary investigations. At 230 nm and 225 nm, chlorantraniliprole and cyantraniliprole were eluted in 4. 9 minutes and 6. 9 minutes, respectively. Relative Standard Deviation (RSD) based on area and retention time were 1. 71 and 0. 05 percent for chlorantraniliprole and 0. 63 and 0. 07 percent for cyantraniliprole, respectively, which were reported to be below the acceptability limits of 5. 0 and 2. 0 percent RSD [6]. A good linearity was obtained with a correlation coefficient (R^2) of 0. 999 for both chlorantraniliprole and cyantraniliprole (Fig. 1, 2, 3 &5). On potato, the LOD and LOQ values for chlorantraniliprole were 0. 021 and 0. 070 µg g-1, respectively, and for cyantraniliprole were 0. 003 and 0. 011 µg g^{-1}. The mean recovery for chlorantraniliprole was 97. 16, 97. 28 and 97. 74 per cent with RSD percentage of 2. 48, 2. 28 and 2. 37 respectively, and for cyantraniliprole, the mean recovery percentage was 95. 34, 95. 77 and 98. 01 percent with RSD percentage of 2. 66, 2. 41 and 2. 47 respectively from samples fortified at 0. 05, 0. 25 and 0. 5 ppm (Table 1&2; Figure 4&6). The method's suitability for residue analysis has thus been demonstrated, with a recovery range of 60 to 140 percent [11]. Chlorantraniliprole 18. 5 S @ 30 g a. i. ha-1 and cyantraniliprole 10. 26 OD @ 75 g a. i. ha-1 as foliar spray residues in potato were below detectable levels (BDL) (Table 3). The time between the last insecticidal spray and the harvesting of the potato tuber was approximately 35 days. This could be the cause of chlorantraniliprole and cyantraniliprole degradation in potato tubers.

Cowpea showed an initial deposit of 0. 55 mg kg^{-1} of chlorantraniliprole residues with a half-life of 1. 31 days and a waiting period of 0. 62 days [12]. The half-life of chlorantraniliprole was determined to be 6. 55~11. 49 days in soil and 3. 82~10. 70 days in tomato, according to QIN *et al*. (2010) [13]. Preethi *et al*. (2019) also indicated that more than 80% of chlorantraniliprole was evaporated and recorded at a below detectable level at 5 and 7 days after spraying on cabbage [14]. The final residual of chlorantraniliprole in tomatoes was less than 0. 3 mg kg^{-1}. The half-life ($t_{1/2}$) of chlorantraniliprole in tomato fruit and soil was 3. 30 and 3. 66 days, respectively, according to Malhat *et al*. (2012) [15]. HONG *et al*. (2017) reported that when 10 percent cyantraniliprole OD was given at 42 g a. i. /hm^2 at double the permissible dosage, the half-life of cyantraniliprole in *Brassica oleracea* was 3. 86 days [16]. The potato samples collected from cyantraniliprole at 75 and 150 g a. i. ha^{-1} treated plots were recorded below detectable level (BDL) at the time of harvest for residue analysis, according to Bojan *et al*. (2021) [17]. Malhat *et al*. (2018) reported that harvest time residues of tomato sprayed with cyantraniliprole ranged from 0. 105 to 0. 196 mg/kg and 0. 170 to 0. 194 mg/kg, respectively, with a half-life of 2. 6 days [18]. According to Hu *et al*. (2013), the half-life of cyantraniliprole in watermelon and soil in Zhejiang was 1. 1 and 4. 1 days, respectively, and 2. 7 and 2. 6 days in Hunan [19].

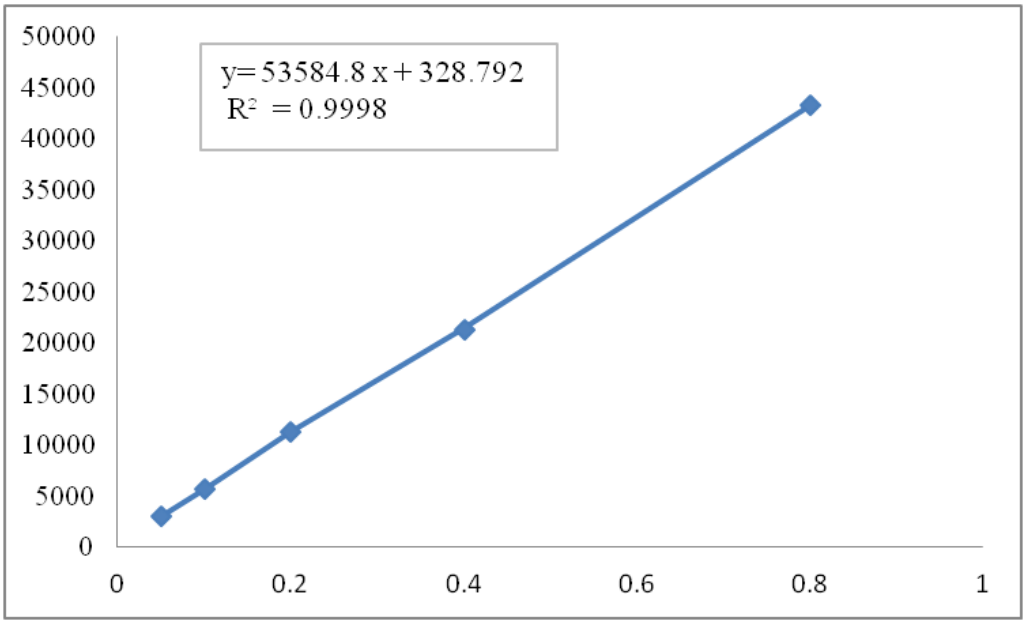

Fig 1: Calibration curve of chlorantraniliprole in UHPLC

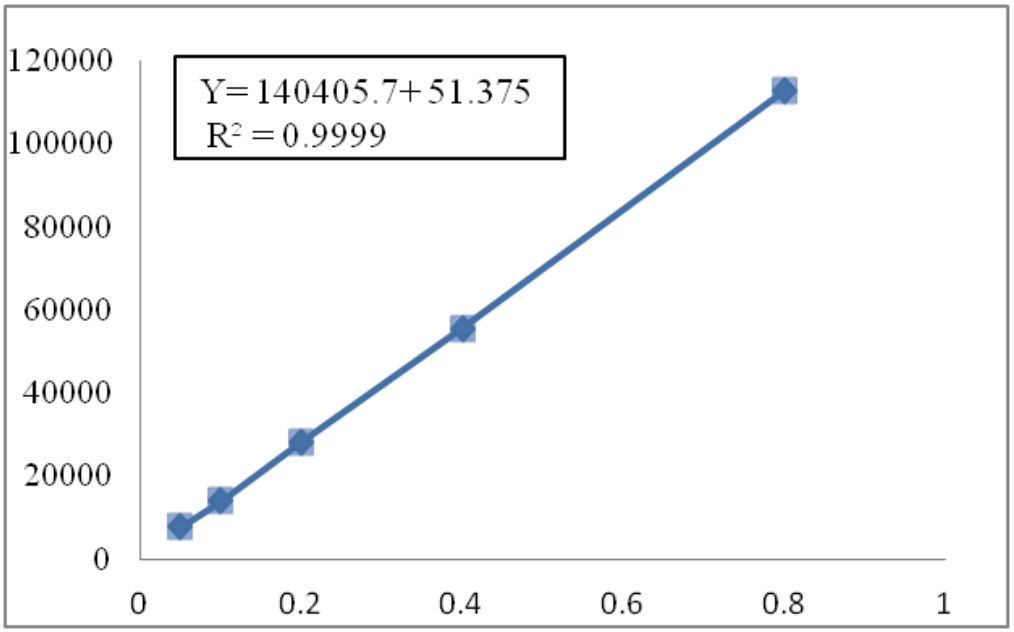

Fig 2: Calibration curve of cyantraniliprole in UHPLC.

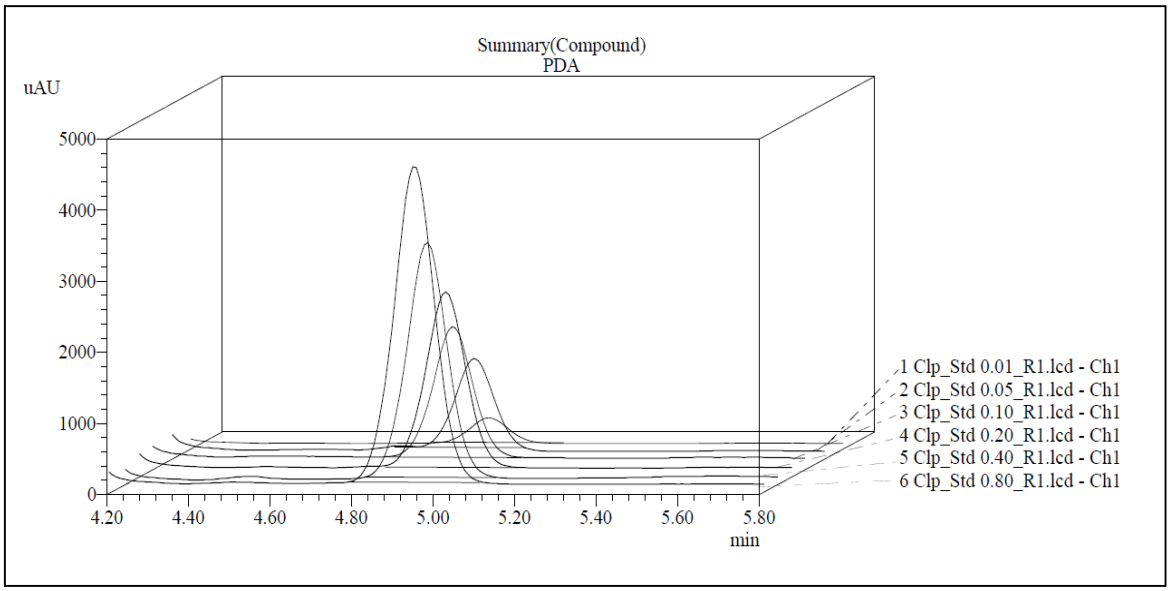

Fig 3: Linearity curve of chlorantraniliprole in UHPLC.

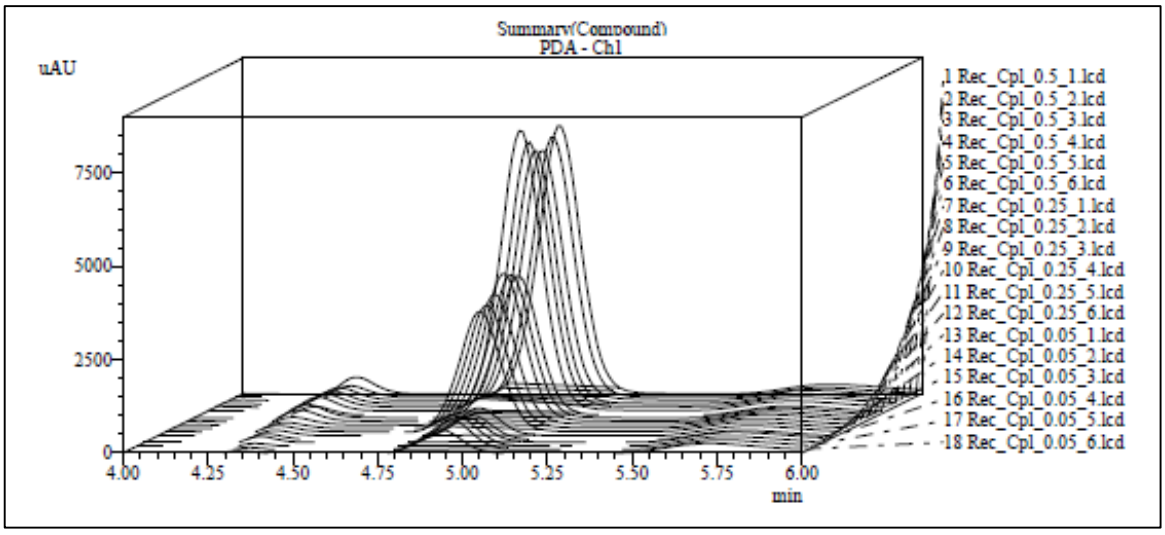

Fig 4: Recovery curve of chlorantraniliprole in UHPLC.

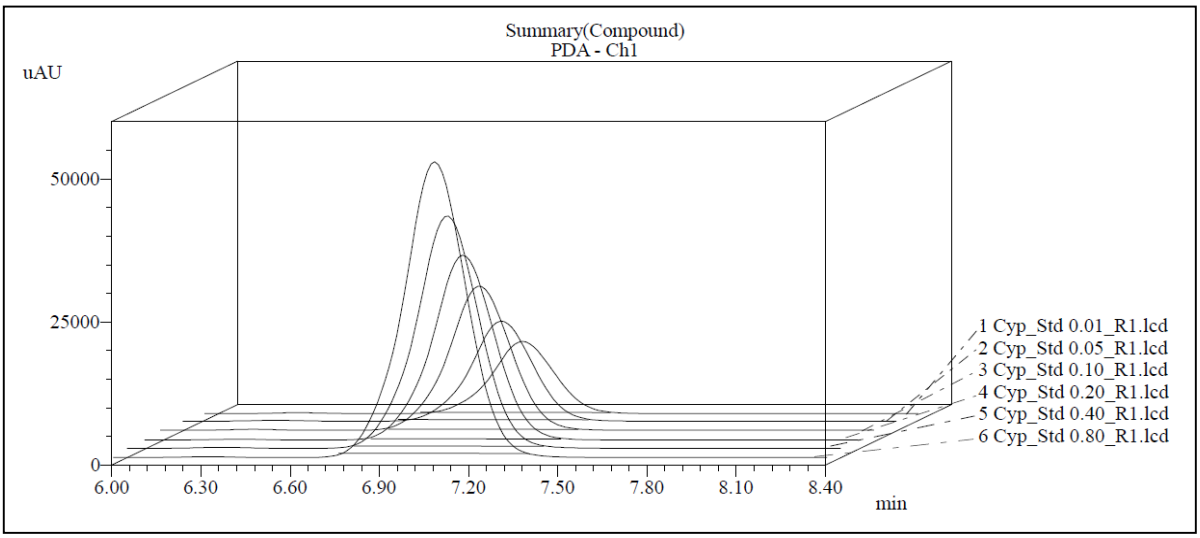

Fig 5: Linearity curve of cyantraniliprole in UHPLC.

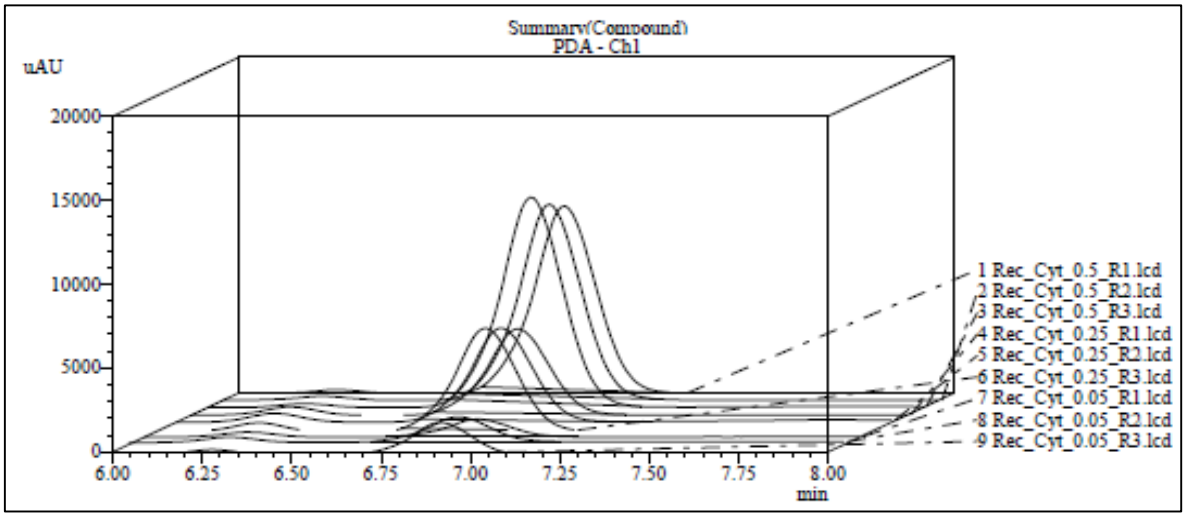

Fig 6: Recovery curve of cyantraniliprole in UHPLC.

Table 1: Recovery percentage of chlorantraniliprole at different fortified levels in/on potato

Fortified concentration (µg/g)	Recovery (%)							
	R1	R2	R3	R4	R5	R6	Mean* (%) ± SD	% RSD
0. 05	92. 25	98. 98	98. 65	98. 28	98. 79	96. 02	97. 16± 2. 410	2. 480
0. 25	99. 08	98. 34	99. 58	97. 97	95. 41	93. 30	97. 28± 2. 217	2. 279
0. 5	92. 90	98. 63	98. 83	99. 13	99. 83	97. 08	97. 74±2. 313	2. 366

*Mean of six replicates; SD – Standard Deviation, RSD- Relative Standard Deviation

Table 2: Recovery percentage of cyantraniliprole at different fortified levels in/on potato

Fortified concentration (µg/g)	Recovery (%)				
	R1	R2	R3	Mean* (%) ± SD	RSD(%)
0. 05	93. 41	94. 40	98. 21	95. 34± 2. 532	2. 656
0. 25	93. 60	95. 52	98. 19	95. 77± 2. 307	2. 409
0. 50	95. 28	99. 91	98. 83	98. 01± 2. 423	2. 472

*Mean of three replicates; SD – Standard Deviation, RSD- Relative Standard Deviation

Table 3: Harvest time residue of chlorantraniliprole 18. 5 SC and cyantraniliprole 10. 26 OD in potato

Insecticide	Dosage in g a. i. h^{-1}	Residue in µg g^{-1}
Chlorantraniliprole 18. 5 SC	30	BDL
Cyantraniliprole 10. 26 OD	75	BDL
Untreated control	-	BDL

4. Conclusion

To summarise, a method for chlorantraniliprole and cyantraniliprole was developed and validated in UHPLC using a PDA Detector, with high specificity. To validate the method for pesticide residue analysis, the following parameters were calculated: specificity, linearity, recovery, repeatability, and ruggedness. In UHPLC with a PDA detector, the LOD and LOQ values for chlorantraniliprole were 0. 021 and 0. 070 µg g^{-1}, respectively, and for cyantraniliprole, 0. 003 and 0. 011 µg g^{-1} on potato. The residues of cyantraniliprole and chlorantraniliprole in potato tubers recovered from treated plots at harvest were recorded at a level that was below detectable.

5. References

1. Lahm GP, Selby TP, Freudenberger JH, Stevenson TM, Myers BJ, Seburyamo G *et al*. Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. Bioorganic & Medicinal Chemistry Letters. 2005;15(22):4898-906.
2. Pubchem. https://pubchem.ncbi.nlm.nih.gov/ compound/Chlorantraniliprole. 20 may, 2019.
3. EPA. Pesticide Fact Sheet. Chlorantraniliprole, Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, United States. [Online]. http://www.epa.gov/opprd001/factsheets/chloran. pdf. April, 2008.
4. Hannig GT, Ziegler M, Marçon PG. Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode- of- action groups. Pest Management Science: formerly Pesticide Science 2009; 65(9):969-74.
5. Selby TP, Lahm GP, Stevenson TM, Hughes KA, Cordova D, Annan IB *et al*. Discovery of cyantraniliprole, a potent and selective anthranilic diamide ryanodine receptor activator with cross-spectrum insecticidal activity. Bioorganic & medicinal chemistry letters 2013;23(23):6341-5.
6. SANTE. The European Commission, 2017. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. Document no. SANTE/11813/2017, 47. http://www. crl-pesticides. eu/ docs/public/tmplt_article. asp?CntID=727&LabID=100&Lang=EN.
7. Ellison SL, Williams A. Quantifying uncertainty in analytical measurement 2012.
8. Bertil M, Örnemark U. The fitness for purpose of analytical methods: A laboratory guide to method validation and related topics. A laboratory guide to method validation and related topics, LGC, Teddington, Middlesex, UK 2014.
9. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC international 2003;86(2):412-31.
10. Paramasivam M, Banerjee H. Dissipation of flubendiamide residues in/on cabbage (Brassica oleracea L.). Environmental monitoring and assessment. 2013;185(2):1577-81.
11. Thompson M, Ellison SL, Wood R. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure and Applied Chemistry 2002;74(5):835-55.
12. Vijayasree V, Bai H, Beevi SN, Mathew TB, Kumar V, George T *et al*. Persistence and effects of processing on reduction of chlorantraniliprole residues on cowpea fruits. Bulletin of environmental contamination and toxicology 2013;90(4):494-8.
13. Qin DM, Qin X, XU YM, Sun Y, Liang XF, Dai XH. Residue determination and degradation of chlorantraniliprole in soil and tomato. Journal of Agro-Environment Science 2010, 5.
14. Preethi S, Vinothkumar B, Bhuvaneswari K, Paramasivam M. Dissipation pattern of chlorantraniliprole in/on cabbage. International Journal of Chemical Studies 2019;7(3):4791-5.
15. Malhat F, Abdallah H, Hegazy I. Dissipation of chlorantraniliprole in tomato fruits and soil. Bulletin of environmental contamination and toxicology 2012; 88(3):349-51.
16. Hong W, Wu Y, Wei J, Wu Y, Soil FS, Zhejiang P. Efficiency of cyantraniliprole on *Plutella xylostella* (L.) and its residual dissipation dynamics in Brassica oleracea. Chin J Pestic Sci 2017;19(2):211-6.
17. Bojan V. Bioefficacy, phytotoxicity, safety to natural enemies and residues of cyantraniliprole 10 OD on potato (*Solanum tuberosum* L.) under open field condition. Crop Protection 2021;142:105505.
18. Malhat F, Kasiotis KM, Shalaby S. Magnitude of cyantraniliprole residues in tomato following open field application: pre-harvest interval determination and risk assessment. Environmental monitoring and assessment. 2018;190(3):1-0.
19. Hu X, Zhang C, Zhu Y, Wu M, Cai X, Ping L, *et al*. Determination of residues of cyantraniliprole and its metabolite J9Z38 in watermelon and soil using ultra-performance liquid chromatography/mass spectrometry. Journal of AOAC International 2013;96(6):1448-52.