Validation and monitoring of chlorantraniliprole residues in polished and parboiled rice using LC-MS/MS

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ABSTRACT

Effective analytical method was developed and validated for the detection and quantification of chlorantraniliprole insecticide residues in polished and parboiled rice samples using a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method and liquid chromatography mass spectroscopy/mass spectroscopy (LC-MS/MS). The method was validated by linearity, limits of detection, limit of quantification, precision and accuracy. At concentrations between 0.01 and 0.1 µg g^{-1}, chlorantraniliprole exhibited good linearity in the matrix, with a correlation coefficient (R^2) > 0.98 and a slope ratio showing a matrix attenuation effect. The limits of detection and limits of quantification were 0.004 µg g^{-1} and 0.012 µg g^{-1} for polished and parboiled rice. The recovery study was conducted at five different fortification levels ranging from 0.01 to 0.1 µg mL^{-1}. Chlorantraniliprole recovery rates varied from 80.91 to 106.84 and 91.17 to 99.99 percent for polished and parboiled rice, respectively. The current method was found to be quick, easy, accurate, and reliable, making it the perfect analytical strategy for finding residues of chlorantraniliprole in rice samples. Monitoring of super market samples revealed that, out of 30 samples of polished and parboiled rice, none of the samples were contaminated with chlorantraniliprole residues.

Keywords: Polished rice, parboiled rice, residue, QuEChERS, LC-MS/MS, monitoring

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Short running title: Validation and monitoring of chlorantraniliprole residues in polished and parboiled rice samples through LC-MS/MS.

INTRODUCTION

Rice is grown as a staple crop in six continents and in the Indian states of Tamil Nadu, Punjab, Kerala, Karnataka, Orissa, Andhra Pradesh, and Gujarat (Prasad *et al*., 2017; Singh *et al.*, 2014). India ranks second in the world in terms of production of rice (163.52 MT) and has the largest rice farming area (43.5 Mha) (Anonymous, 2020). Several biotic and abiotic components operate as yield restrictions in rice, with insect-pests causing a significant yield loss in rice production and productivity (Chatterjee *et al*., 2016). In India, more than 100 insect species attack rice, with approximately twenty of them considered serious pests, causing up to 30% crop loss (Salim *et al*., 2001). Yellow stem borer, *Scirpophaga incertulas* Walker, and rice leaf folder, *Cnaphalocrocis medinalis* Guenee, are the most frequent and damaging insect pests in the country, producing output losses of 10-60% (Chatterjee and Mondal, 2014). Pests that destroy crops have forced farmers to apply pesticides close to harvest in numerous cases. In order to sustain crop yield potential and assure a sufficient agricultural production, pesticide spraying is viewed as a critical management approach (Tindall *et al*., 2005).

Chlorantraniliprole (3-Bromo-N-[4-chloro-2-methyl-6[methylamine] carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1Hpyrazole-5 carboxamide) is a plant systemic pesticide that interferes with normal muscle activity (Lahm *et al*., 2007). Insect ryanodine receptor activation induces an excessive release of calcium (Ca2+) from sarcoplasmic reticulum muscle cells, impairing muscular paralysis, causing feeding to stop, lethargy, and ultimately insect mortality (Cordova *et al*., 2006). It is marketed as chlorantraniliprole 18.5% SC (Suspension Concentrate) and 0.4% G (Granules), both of which are recommended for use in paddy against yellow rice stem borer and leaf folder (Lahm *et al*., 2005).

The majority of research on chlorantraniliprole has focused on its chemical synthesis, effectiveness, toxicity, and mode of action (Teixeira *et al*., 2009). However, numerous analytical approaches for identifying and quantifying chlorantraniliprole residues in diverse crop ecosystems, such as fruits, vegetables, pulses, and cereals, have been mentioned. HPLC with a

photodiode array detector was used to analyze chlorantraniliporle residue in grape and tomato samples (Malhat *et al*., 2012), and HPLC with a mass spectrometer in corn (Dong *et al*., 2011), maize (He *et al*., 2016), and rice (Xu *et al*., 2010; Zhang *et al*., 2012; Mahato *et al*., 2023). The extraction and cleanup processes of an existing approach were optimized in this study. To our knowledge, only few investigations on pesticide residue monitoring in various paddy matrices, such as paddy straw, grain, and processed products, have been conducted. The purpose of this work was to establish a method for assessing chlorantraniliprole residues in polished rice and parboiled rice using LC-MS/MS, as well as to monitor the residues in super market-collected samples.

MATERIALS AND METHODS

The chlorantraniliprole reference standard (97.63%) was bought from Dr. Ehrenstorfer in Augsburg, Germany. The MS-grade formic acid and acetonitrile were bought from Sigma Aldrich in Mumbai, India. Merck India Ltd. (Mumbai, India) supplied the magnesium sulfate and anhydrous sodium chloride, which were warmed at 650°C for four hours before use. From M/s. Agilent Technologies (Agilent Technologies India Pvt Ltd, Chennai, India), primary secondary amine (PSA) was purchased. The technical-grade substance (10.4 mg) was dissolved in acetonitrile (v/v) to make a stock solution (400 μg mL^{-1}) of chlorantraniliprole (97.63%). This was labeled and stored in a freezer at -20°C. The stock solution was used for making the working standard solution and intermediate stock solution. A Merck Millipore device was used to obtain ultrapure water. The sample extracts were filtered using nylon filters (0.2 μm) from PAL Life Sciences (PALL Life Sciences, Bangalore, India).

The Waters Alliance 2695 Separation system, which includes an autosampler, quaternary pump, and membrane degasser, was used for the liquid chromatographic (LC) analysis. An acquity TQD was linked for mass spectrometry using an ESI interface. For the analyte separation, a 5 m (4.8 x 250 mm) X Terra analytical C18 column was employed. The temperature of the column was fixed at 30°C. Following, an isocratic flow of 30% A + 70% B at 0.5 mL min^{-1}. To evade the analyte, a 10 μL injection volume and a 10-minute run were utilized.

The desolvation (drying gas) and cone gas flows were set at 1100 and 80 L h^{-1} and 0.20 mL min^{-1}, respectively, while the source block and desolvation temperatures were set at 150 and

500^{o}C and respectively. At 3.8 kV, the capillary voltage was set. The voltages for the cone, extract, and RF lenses were 45, 5, and 0.2 V, respectively. The precursor ion intensity was increased using these interface conditions. Argon served as the collision gas, while nitrogen served as the desolvation and cone gases.

The paddy whole grain samples were collected from organic fields of Department of Sustainable Organic Agriculture in Coimbatore, Tamil Nadu, India. For parboiled rice, paddy whole grains were soaked in hot water for three hours at 70°C. After that, water was drained from samples and steamed for 15 min. Finally, samples were dried under sun for 3 hours (Shakuntala and Shadaksharaswamy, 2001). Dehusking of paddy whole grains were done using a rice sheller. Unpolished rice was passed through rice polisher for polished rice. Unpolished rice and parboiled rice were homogenized using a high-speed blender, processed into a fine powder, and kept at 4°C in a freezer.

For sample extraction and cleaning, the QuEChERS approach (Anastassiades *et al*., 2003; Karthikeyan *et al*., 2019) was utilized with a few modifications. 10 g of polished/ porboiled rice powder was placed in a 50 mL centrifuge tube. To this, 10 mL of distilled water was added and moister for 20 min. Then, 20 ml of acetonitrile was added to the centrifuge tube and vortexed for one minute. Four g of anhydrous magnesium sulphate and 1 g of sodium chloride were added to this, and the sample was thoroughly mixed for a minute by vortex shaking. For 10 minutes, the samples were centrifuged at 6000 rpm. The supernatant was then run through 4 g of anhydrous sodium sulphate to eliminate any leftover moisture. Of this, 6 mL was placed in a centrifuge tube along with 100 mg of PSA and 600 mg of anhydrous magnesium sulphate. These materials were fully homogenized by vortexing for one minute and centrifuging at 3000 rpm for ten minutes. Finally, 4 mL of the extract was placed in a glass test tube and concentrated nitrogen gas in a Turbovap (Caliper life sciences, USA) at 40 °C until it was close to dry. The final volume was reconstituted with acetonitrile to 1 mL, filtered over a 0.2 µm filter membrane, and placed in a 1.5 mL glass auto sampler vial for LC/MS-MS analysis.

By evaluating the method performance in accordance with SANTE, 2021 standards, the analytical technique utilized to determine pesticide residues in polished and parboiled rice was validated in the lab. By calculating and assessing a number of performance criteria, including linearity, limit of detection (LOD), limit of quantification (LOQ), matrix effect, recovery, and

precision, the analysis technique utilized in the current study was confirmed. The linearity of the detector response for the test analyte was assessed by injecting six calibration working standard solutions in LC-MS/MS at concentrations of 0.003, 0.01, 0.025, 0.05, 0.075, and 0.1µg mL^{-1} with three replicate injections per concentration.

Following seven replications, samples of spiked polished and parboilrd rice were injected, and the LOD and LOQ were calculated. The LOD and LOQ were determined using the standard deviation, standard error, and regression (SANTE, 2021). The ME (%) method was used to calculate the matrix effect.

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

Untreated (polished and parboiled rice) samples were homogenized before being spiked with the usual chlorantraniliprole solution. After being spiked with the analyte at concentrations of 0.01, 0.025, 0.05, 0.075, and 0.1µg mL^{-1}, the samples were extracted and cleaned. Seven times were repeated for each spiking sample. Based on calculations of analyte value performed during recovery analyses, the relative standard deviation (RSD %) was used to evaluate the procedure's accuracy.

Calculations of the relative standard deviation, matrix effect, recovery percentage, and residue concentration were made using the information from the chromatogram. The method of residue quantification was carried out using the subsequent equation.

$$\text{Residues (µg } g^{-1}) = \frac{As}{Astd} \times \frac{Wstd}{Ws} \times Vs$$

As - Sample peak area

Astd - Standard peak area

Wstd - Weight of standard (µg mL^{-1})

Ws - Weight of the sample (g)

Vs - Volume of the final extract in mL

For this study, samples of polished rice (30 numbers) and parboiled rice (30 numbers) were gathered from super markets of Coimbatore, Salem, and Thanjavur in Tamil Nadu, India. The collected samples were packaged in plastic bags with labels, and taken to the lab for analysis. The samples were examined using the procedure that has been validated above.

RESULTS AND DISCUSSION

Tuning LC-MS/MS in MRM mode by infusing conventional chlorantraniliprole solutions resulted in enhanced fragmentation at collision energies ranging from 2 to 80V. The [M+H]+ parent ion was the normal ESI positive mode ion for chlorantraniliprole. For the analyte chlorantraniliprole, two MRM transitions were followed (482.13> 283.87 and 111.92 m/z), allowing for simultaneous quantification and identification. Using an isocratic run, it was discovered that the optimal combination was 0.1% HCOOH in water and 0.1% HCOOH in acetonitrile in a 30:70 v/v ratio. The analyte studied under the predefined chromatographic conditions revealed good peak shape and separations during a duration of 10 minutes (Figure 1a).

The linearity of the calibration curve for the chlorantraniliprole standard was found to be satisfactory (0.999). There were no background peaks above a signal-to-noise ratio of 3 for the retention duration of the examined analyte, showing that the selective ion monitoring approach was interference-free. The LOD and LOQ for polished and parboiled rice were 0.004 and 0.012 µg g^{-1}, respectively (Table 1). Zang *et al*. (2012) found the LOD of chlorantraniliprole was 0.15 µg kg^{-1} in brown rice matrices using LC–ESI–MS/MS. Mahanto *et al*. (2023) also reported the LOQ of chlorantraniliprole was 0.01 µg g^{-1} for brown rice and these results are in accordance with our results.

The sample matrix could have a considerable impact on the accuracy of pesticide analysis results (Santilio *et al*., 2014). Interferences in the matrix can suppress or amplify analytical data, resulting in poor or high analyte recoveries (Zhang *et al*., 2012). Matrix match standards were used to quantify the analyte in the polished and parboiled rice samples in order to reduce the matrix impact. The matrix effect for chlorantraniliprole residues in polished and parboiled rice samples in this investigation ranged from -4.261 to 8.520 and -11.921 to 11.307 percent,

respectively (Table 1). In polished and parboiled rice samples, suppression of ion response were observed for chlorantraniliprole.

A quantitative analysis must be able to provide mean recovery values between 70 and 120 percent at each level of spiking, according to SANTE (2021). Acetonitrile extraction produced good recoveries of 80.91 to 106.84 and 91.17 to 99.99 percent for polished and parboiled rice respectively, at spiking doses ranging from 0.01 to 0.1 µg mL^{-1} (Table 2). In this study, 100 mg PSA was used for the cleanup of polished and parboiled rice that contained organic and fatty acids, sugars and anthocyanin pigments. Using acetonitrile as an extraction solvent, Zhang *et al*. (2012) observed a satisfactory recovery of chlorantraniliprole in brown rice (84.9 to 87.7%). Mahanto *et al*. (2023) used acetonitrile extraction and obtained good chlorantraniliprole recoveries from brown rice (93.57 to 96.40%), which are comparable to our results.

In a monitoring investigation with 30 samples of polished rice and 30 samples of parboiled rice, no samples were found to be contaminated with chlorantraniliprole residues. MRL for chlorantraniliprole fixed by CODEX for rice and polished rice is 0.4 and 0.04 mg $Kg^{-1.}$ However, pesticide residue was not detected in any of the polished and parboiled rice samples tested.

CONCLUSION

The current study aimed to develop a method (recovery of 70 to 120% and RSD 20%) for determining residues in paddy samples, which are a common source of food in India. A quick, easy, and accurate analytical approach for determining chlorantraniliprole residues in polished and parboiled rice was developed using LC/MS/MS. The present method was validated in accordance with European Union requirements and is also suited for assessing residues in polished and parboiled rice samples.

DECLARATION

Authors declare that they have no conflict of interest

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There is no role of funding in this study design, data collection, analysis, decision to publish, or preparation of the manuscript**.**

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Table 1. Linearity, LOD, LOQ and matrix effect of polished rice and parboiled rice on chlorantraniliprole residues

Matrix	Calibration range (mg L^{-1})	Regression equation	Correlation coefficient (R^2)	Matrix effect (%)	LOD	LOD
Polished rice	0.01-0.1	y = 21905x + 104.2	0.975	-4.261 to 8.520	0.004	0.012
Parboiled rice	0.01-0.1	y = 6037x + 8.558	0.997	-11.921 to 11.307	0.004	0.012

Table 2. Recovery and Precision of chlorantraniliprole in polished rice and parboiled rice samples

Matrix	Spiking level (µg g^{-1})									
	0.01		0.025		0.05		0.075		0.1	
	Recovery (%)		Recovery (%)		Recovery (%)		Recovery (%)		Recovery (%)	
	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD
Polished rice	106.84 ± 7.74	7.24	86.05 ± 4.21	4.89	99.88 ± 6.80	6.81	80.91 ± 4.60	5.69	81.33 ± 5.00	6.15
Parboiled rice	96.07 ± 6.17	6.42	91.17 ± 8.63	9.46	98.15 ± 7.58	7.73	93.67 ± 8.13	8.68	99.99 ± 9.58	9.58

Figure 1. LC-MS/MS Standard chromatogram of chlorantraniliprole (0.01 µg g^{-1})

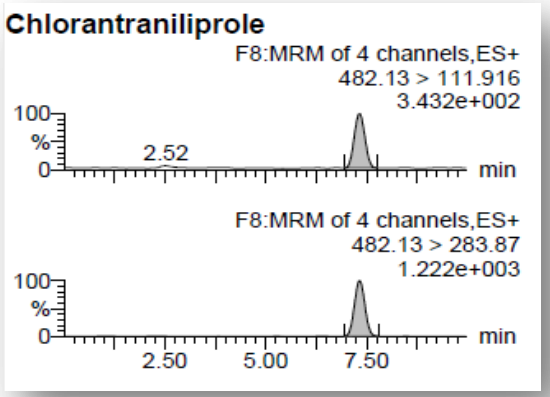
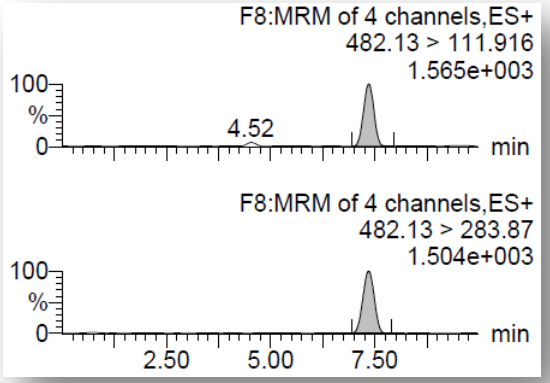
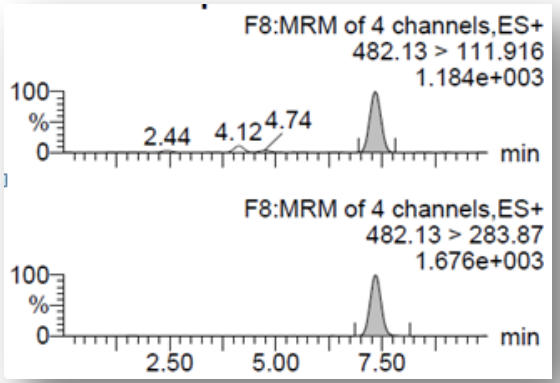


Figure 2. Recovery chromatogram of chlorantraniliprole (0.01mg kg^{-1}) in polished rice (2a) and parboiled rice (2b) using LC-MS/MS



(a)



(b)