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Determination of Tricyclazole Fungicide in Rice using LC-MS/MS and its Risk Assessment

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An analytical method using liquid chromatography tandem mass spectrometry (LC-MS/MS) along with QuEChERS method of sample preparation was developed and validated for the determination of tricyclazole residues in rice. The limit of quantification (LOQ) for the method was 0.002 µg g^{-1}. The average recovery was in the range of 91.21 to 99.75 per cent with relative standard deviation (RSD) of less than 10 per cent for the spiked concentrations at 0.002, 0.01 and 0.02 µg g^{-1}. The method was successfully applied to analyse rice samples collected from the local markets. Residues of tricyclazole were detected in 45 per cent of the samples, of which 31.37 per cent contained it above the maximum residue level (MRL, 0.01 mg kg^{-1}) fixed by Food Safety and Standards Authority of India. Assessment of the human risk suggested that the rice at the contaminated detected levels has minimal contribution to toxicological risks as it recorded hazard index value of less than 1. However, precautionary measures for the consumer safety are suggested. The present method could be adopted for monitoring tricyclazole residues in rice samples.

Keywords: Tricyclazole, rice, LC-MS/MS, residue, QuEChERS, risk assessment

Rice is one of the important staple foods for the growing population in India and other south East Asian countries (IRRI, 2006; Luca Pareja *et al.,* 2011). It is a major cereal grown in over 100 countries, and consumed by over two billion people in the world (Nguyen *et al.,* 2008; Tsochatzis *et al.,* 2010). India produces rice for its indigenous use and export in an area of 44.5 m ha with production of 110.15 mt annually (FAO, 2015). The rice yields (rice production and productivity) have declined due to insect pests, diseases, weeds and various other reasons; among those diseases being the major yield limiting factor with potential to cause major economic loss to the crop. The annual losses due to rice diseases are estimated to be 10-15 per cent on an average basis worldwide (Bonman, 1992; Prasanna Kumar *et al.,* 2013). Rice is attacked by various diseases including the blast caused by ascomycete fungus *Pyricularia grisea* Sacc., (Telemorph: *Magnaporthe grisea*) (Correll *et al.,* 2000). The fungus can infect many parts of the plant but the most damaging phase of the disease is the nodal or panicle infection (Ou, 1985). Use of resistant cultivars, fungicide application and manipulation of date of planting, fertilizers and irrigations are the most usual approaches for the management of rice blast disease (Georgopoulos and Ziogas, 1992; Moletti *et al.,* 1988). Among the various methods of control of the blast disease (Mariappan *et al.,* 1995), chemical control with tricyclazole fungicide is widely practiced in many countries. Seed treatments with systemic fungicides and foliar sprays have been found to be effective in reducing the blast disease incidence (Chaudhary, 1999; Chaudhary and Sah, 1998). Tricyclazole (5-methyl-1,2,4-triazolo[3,4-b][1,3] benzothiazole) is a systemic fungicide, absorbed rapidly by the roots with translocation through plant and used for the control of rice blast in transplanted and direct-seeded rice. It is applied as a drench, transplant root soak

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or foliar application. Different formulations of tricyclazole registered and available are 75 per cent WP, 33.3 per cent SC (Froyd *et al*., 1977).

In India, tricyclazole 75 per cent WP is widely used by the farmers to prevent leaf and neck blast in paddy (Ghimire *et al.,* 2017). However, the continuous and indiscriminate use of tricyclazole has toxic effects on the non-target organisms and can cause unwanted effects in the environment (Alana *et al*., 2017). Tricyclazole residues in rice do not only threaten the health of consumers but also affect the rice export to other countries. For example, India's exports of the aromatic Basmati rice to the European Union came to a halt due to tricyclazole residues which were above the maximum residue level (MRL) (Sen, 2017). In India, the tolerance limit of tricyclazole fungicide in rice is 0.01 mg kg^{-1} (FSSAI, 2018). On the other hand, there is a debate on banning it in the European Union due to the harm it may cause to human health. It is, therefore, essential to monitor the presence of tricyclazole and regulate its levels in rice.

To monitor tricyclazole in rice requires powerful analytical methods which are highly sensitive, selective, accurate and capable of efficiently measuring residues at very low levels (Grimalt and Dehouck, 2016). Traditionally, pesticide residues are analyzed mainly by gas chromatography/mass spectrometry (GC/MS) method (Chen *et al*., 2012), but GC cannot be used for compounds that are thermally labile. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has therefore become the method of choice for pesticide analysis due to its high selectivity and sensitivity as well as its suitability *viz.,* in pigeonpea for indoxacarb analysis (Naik *et al.,* 2020) and for a wide range of compounds in various sample matrices (Tomas Cajkaa *et al*., 2012; Zhao *et al*., 2012) and the features of LC-MS such as efficient separation, precise identification and quantification of analytes at trace levels have made this technique adoptable for monitoring of pesticide residue in agricultural produce and processed products. In addition, an important aspect of MS is the ability to perform confirmation and quantification of compounds of the same class as well as compounds lacking a chromophore. Furthermore, the MS response is not limited by spectra overlap which is a unique advantage of LC-MS. This allows the determination of a broad spectrum of pesticides and hence, efficiency in pesticide residue analysis.

During the past few years several analysts have adopted chromatographic techniques for tricyclazole analysis in food matrices *viz.,* Wu *et al*., 2018 in rice bran protein powder using LC-HRMS; Zhou *et al*., 2013 in corn, barley, brown rice, and oat; Pareja *et al*., 2012 in paddy rice, brown rice and rice bran; Meng *et al*., 2018 in raw and polished rice and Gosetti *et al*., 2014 in water. QuEChERS extraction method has been widely applied for extraction of multi-residue for analysis in food samples including rice (Andrey *et al.,* 2014).

Acute toxic effects of tricyclazole pesticide on animals and humans are easily recognised, but the effects that result from long-term exposure to low doses are often difficult to distinguish. In particular, the effects of a regular intake of pesticide residues in food are hard to detect and quantify. Earlier studies have analyzed the presence of tricyclazole residue in rice grains at higher limit of quantification (LOQ) levels than the present Indian MRL of 0.01 mg kg^{-1}. In Iran, a study involving the simultaneous determination of 41 LC-amenable pesticides belonging to different chemical classes along with tricyclazole in rice by LC-MS/MS has been developed with LOQ and limit of detection (LOD) for tricyclazole as 0.25 and 0.008 µg g^{-1} respectively. It was reported effective in monitoring domestic and imported rice samples at concentrations of 0.0056 to 0.0740 µg g^{-1} (Shakouri *et al.,* 2014). The previously reported studies on residues of tricyclazole in rice by Min *et al*., 2012; Navarro *et al.,* 2011; Pareja *et al.,* 2012; Shakouri *et al.,* 2014 and Zhou *et al.,* 2013 showed higher quantification level of tricyclazole in rice and in longer time programme. However, the reported method in the present study was able to detect and quantify the tricyclazole residue through LC-ESI-MS/MS in rice within a short period of time of 10 min at very low level of 0.002 mg kg^{-1} which is five times lower than Indian MRL of 0.01 mg kg^{-1}.

An exposure or risk assessment is necessary in order

to ascertain the effects due to regular intake of pesticide residues in food. No published data in Indian scenario is available on the levels of tricyclazole residue in rice. This work, therefore, seeks to provide the baseline information on the levels of tricyclazole residue in rice samples collected from North Eastern part of Karnataka and to elucidate their hazard indices. This study aimed to develop and validate a fast, highly sensitive, accurate and selective analytical method for analysis of tricyclazole pesticide in rice samples along with an assessment of its potential risks to consumers.

MATERIALS AND METHODS

Chemicals and reagents

Tricyclazole (>99.0 % purity) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). All reagents were of analytical grade. LC-MS grade acetonitrile and methanol ($\geq$ 99.9% purity) were purchased from J.T.Baker (NJ, USA), ethyl acetate ($\geq$ 99.8% purity) was procured from Merck (Mumbai, India); anhydrous magnesium sulphate (99 % purity), anhydrous sodium sulphate (99 % purity), sodium chloride ($\geq$ 99 % purity), primary secondary amine (PSA, AR grade, particle size of 40μm) were obtained from Agilent Technologies India Pvt. Ltd (Bangalore, India), ammonium formate ($\geq$ 96 % purity) and formic acid ($\geq$ 90% purity) were purchased from Empart (Hyderabad, India). HPLC grade water was generated through a Millipore Milli-Q system.

Preparation of standard solution

A standard stock solution of tricyclozole was prepared by weighing 10.025 mg, transferred into a 10 mL calibrated class A volumetric flask and dissolved in 10 mL of methanol resulting in a final concentration of 992.48 µg mL^{-1}. An intermediate standard solution of 100 µg $mL^{-1}$was prepared by drawing 1.007 mL of stock solution and transferring into a 10 mL volumetric flask and volume made up with methanol. Working standard solutions were prepared from the intermediate stock solution by serial dilution to obtain 0.001, 0.002, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 µg mL^{-1}. The standard solutions were stored in a deep freezer at –20^{o}C.

Sample preparation and extraction of tricyclazole

QuEChERS method and its modification described by Anastassiades *et al.* (2003) and Lehotay *et al.* (2005) were adopted for extraction and clean-up of tricyclazole from an rice grains. The rice (1 kg) sample was collected from an organic shop from Raichur to ensure it to be free from tricylazole residue. The sample was homogenized by using a high volume homogenizer. A test portion of the sample (5 g) was weighed into 50mL centrifuge tube, added 10 mL of each of chilled distilled water and acetonitrile. The tube was capped well and shaken with hand for a min and than the sample was homogenized at 12000 rpm for 2 min. Thereafter, 1.5g NaCl was added to the tube, mixed well by shaking vigorously and centrifuged for 5 min at 12000 rpm to separate the organic layer. The organic supernatant (8 mL) was carefully transferred into a test tube and 3 g anhydrous Na_2SO_4 added to remove moisture. The clean-up was done through dispersive solid phase by weighing out 0.10 g PSA sorbent and 0.30 g anhydrous $MgSO_4$ into 15 mL centrifuge tubes for 6 mL organic layer (extract). Then the tubes were centrifuged for 5 min at 10000 rpm and 1 mL of supernatant was transferred to test tube and evaporated to dryness in turbovap. The residue was reconstituted in 2 mL methanol and filtered through 0.22 µm PTFE filter prior to LC-MS/MS analysis. For the preparation of matrix matched calibration standards (MMC), suitable quantity of standard was added to the control rice matrix in the final reconstitution step.

LC-MS/MS analysis

A Shimadzu 8040 series UHPLC system consisting of a solvent degassing unit, a quaternary pump, an auto-sampler and a thermostated column compartment was used in the LC-MS/MS system. Separation of the analyte was achieved on a Shimpack XR ODS C_{18} column (150 x 2 mm i.d.) with a column oven temperature of 40^{o}C. The mobile phase A consisted of 0.0314g ammonium formate (5mM) + 2mL MeOH + 10µL formic acid (0.01%) and the volume made up with HPLC water to 100mL, and the mobile phase B contained 0.0314g ammonium formate (5mM) + 10µL formic acid (0.01%) and volume made-up with 100 per cent MeOH to 100mL, and delivered at a flow rate of 0.4mL min^{-1}. The tricyclazole was separated with the

following gradient programme: 5 per cent B at start for 0.25 min followed by 100 per cent up to 7.75 min. and then 5 per cent of B for 2 min. Full scan mass spectra of tricyclazole with electro-spray ionization positive mode (ESI^+) was recorded in order to select the most abundant m/z value. Further, the protonated molecular ion $(M+H)^+$ was determined and chosen as the precursor ion. The multiple reaction monitoring (MRM) transitions and associated acquisition parameter were optimised for the maximum abundance of fragmented ions under ESI positive mode. The MS source conditions were as follows: interface voltage of 4.5 kV, desolvation temperature of 250°C, heat block temperature of 400°C, desolvation gas (N_2) flow of 2.9 L min^{-1} and drying gas flow at 2.9 L min^{-1}. Then dissociation with argon was induced and different collision energies were tested in order to find the most abundant product ion. LabSolution® Version 1.5 software was used for the control of equipment, data acquisition and analysis. Optimized transitions used for MS/MS analysis are presented in Table 1.

Table 1. ESI-MS/MS mass spectrometer parameters

Pesticide	RT (min)	Precursor ion (m/z)	Product ions (m/z)	CE (V)
			163.00	24.0
Tricyclazole	3.060±0.1	189.85	136.00	29.0
			109.00	38.0

CE = collision energy (V); RT = Retention time

Method validation

The method of extraction, clean-up, detection and quantification of tricyclazole from rice matrix were optimised and validated in compliance with the European Commission guidance document SANTE/11813/2017 by evaluating the performance parameters: linearity, matrix effect, limit of detection (LOD), limit of quantification (LOQ), specificity, trueness (bias), precision in terms of repeatability (RSD_r), precision in terms of reproducibility (RSD_{wR}) and robustness.

Linearity of the method was assessed in both solvent and matrix matched samples by evaluating the deviation of each concentration injected from the actual linear concentrations (0.002, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 µg g^{-1}), on the basis of linear regression equation. Matrix effect was assessed by comparing the response produced from the analyte in a solvent solution of 0.04 µg g^{-1} with that obtained from the same quantity of analyte in the sample extract. The matrix effect was calculated by comparing the angular coefficients obtained by the calibration curves in the solvent and in the matrix as follows:

$$\text{Matrix effect (\%)} = (b_m - b_s)/b_s \times 100$$

Where, b_m and b_s are the angular co-efficients of the curve in the matrix and in the solvent, respectively (European Commission, 2017; Silva *et al.,* 2012).

The limit of detection (LOD) was experimentally determined by spiked blank rice extracts with tricyclazole. The LOD was calculated by preparing different solutions with low concentration that is expected to produce a response that is 3 times the baseline noise. LOQ was determined in the same manner and is the concentration of pesticide that gives a S/N ratio of 10 and recovery of lowest spike level within the range of 70-120 per cent and a RSD $\leq$ 20 per cent. Measurement uncertainty (MU) was assessed according to International Organization for Standardization (ISO) (Alder *et al.,* 2001 and Eurachem, 2012).

Trueness or bias of the developed method was evaluated by estimating the average recovery for each spiked level tested. Recovery experiments were carried out at 3 fortification levels (0.002, 0.01 and 0.02 µg g^{-1}) by spiking blank rice with working solution of the standard. Spiking was done such that the fortification level is at LOQ level, and 5 and 10 times the LOQ with six replications for each level. The spiked rice samples of six replications at each level were then kept at room temperature of 25°C for 2 h for sample equilibration. The fortified samples were processed as per the previously described sample extraction procedure.

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The method precision was performed with regards to the repeatability relative standard deviation (RSDr). The rice blank samples were spiked with tricyclozole at 0.002, 0.01 and 0.02 µg g^{-1} and extracted. The recovery and RSDr were calculated. Whereas, the RSD with respect to reproductivity (RSDWR) was performed by spiking the tricyclazole in rice blank samples and extracted at two different dates.

Monitoring of fungicide

A total of 51 rice samples were collected from various retail markets of north eastern region of Karnataka. The samples included 41 normal rice, 9 basmati rice and 1 organic rice sold by traders, rice millers and an organic shop. All the rice samples were brought to the laboratory and analysed. The collected samples were processed as per methodology reported.

Health risk assessment

The health risk assessment was carried out by calculating the estimated daily intake (EDI) using hazard index (HI) model (Eissa *et al*., 2014). The EDI (mg $kg^{-1}d^{-1}$) which is a realistic toxicological criterion for the pesticide exposure, was calculated for tricyclazole residue in adults (mean human body weight 60 kg), according to international guidelines by multiplying the average residual pesticide concentration (mg kg^{-1}) by the food consumption by the adult. (In India, it is 0.201 kg d^{-1}, FAO, 2015). The EDI of tricyclazole was then compared with the corresponding acceptable daily intake (ADI) of 0.04 mg kg^{-1} bw established by Global Agriculture Information Network (GAIN, 2004), to calculate the hazard index = (EDI /ADI). If the hazard index (HI) is more than 1, the food is considered as not safe for human consumption (Darko and Akoto, 2008).

RESULTS AND DISCUSSION

LC-MS/MS method development

A validated, selective and sensitive LC-MS/MS method has been developed for the analysis of tricyclazole fungicide having importance in rice export trade. The chromatographic conditions *viz.,* mobile phase flow rate of 0.4mL $min.^{-1}$, LC time programming for 10 min and injection volume of 2 µL were optimized with C-18 column and a gradient programme for 10 min produced the best sensitivity and good peak shape (Fig 1a). A full scan mass spectrum of tricyclazole was recorded in order to select the most abundant m/z value. For the analyte, the protonated molecular ion $(M+H)^+$of 189.85 was determined and chosen as the precursor ion. The multiple reaction monitoring (MRM) transitions and associated acquisition parameters were optimised for the maximum abundance of fragmented ion under ESI positive mode by injecting 2 µL of 0.01µg mL^{-1} tricyclazole compound into the tandem mass spectrometer. The dissociation with argon was induced and different collision energies were tested in order to find the most abundant product ion. The optimised precursor and product ion transitions used for MS/MS analysis and the collision energies of tricyclazole are shown in Table 1 and Figure 1b. All the three product ions *viz*., 163.00, 136.00 and 109.00 were used for the quantification of tricyclazole.

The developed LC-MRM mode provides high sensitivity and selectivity requirements for analytical method used for the detection of tricyclazole at low level concentration from 0.002 mg kg^{-1}in the rice matrix. Under the developed method, tricyclazole was found to show a peak at a retention time of 2.726±0.1 min. (Fig. 1a). The total and extracted ion chromatograms of tricyclazole standard are shown in Figures 1a and 1c, respectively.

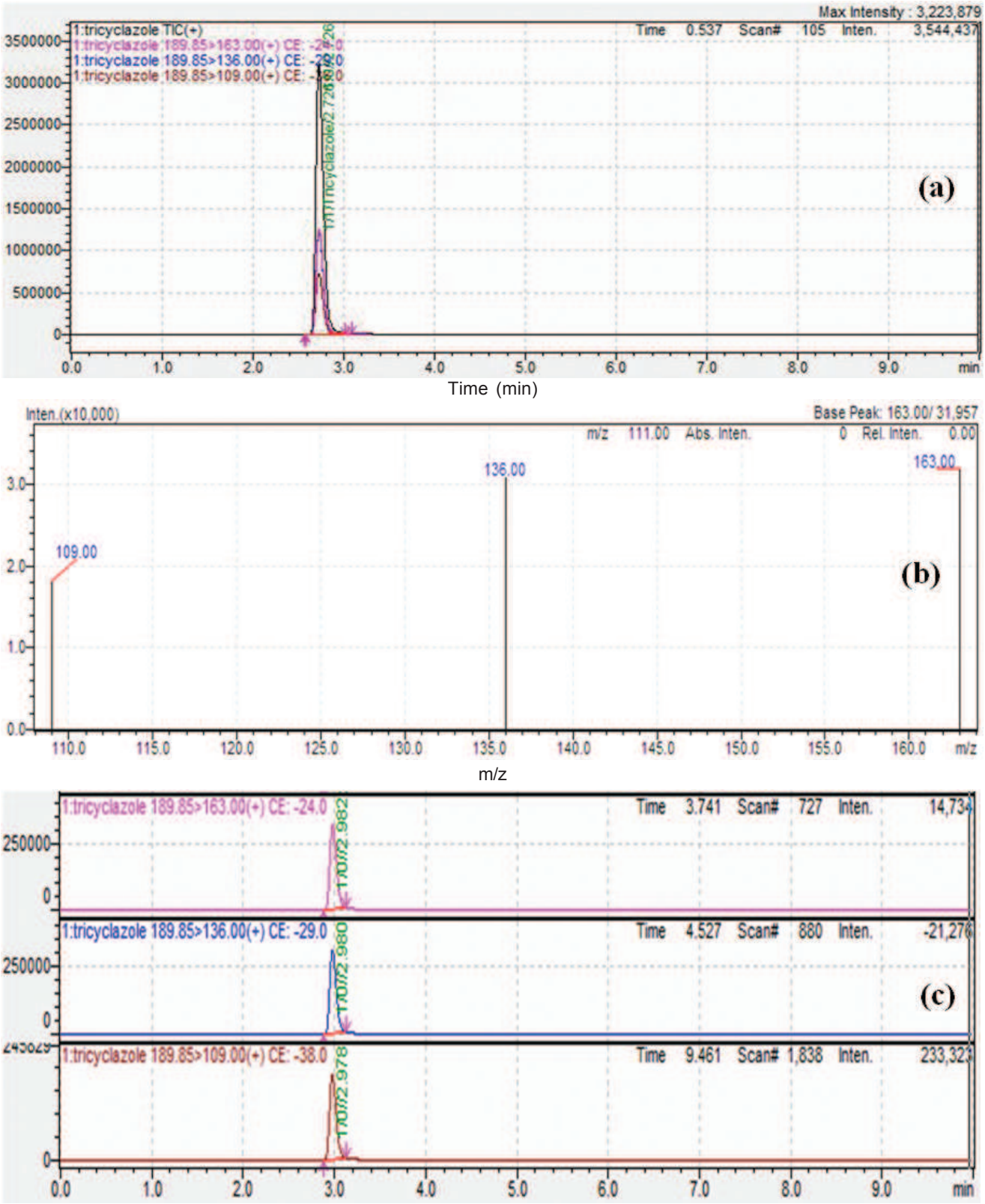

Figure 1: Tricyclazole total ion chromatogram along with product ions (a), multiple reaction monitoring transitions of tricyclazole (b), and chromatogram of tricyclazole depicting different product ions

Method validation

The optimised analytical method was validated according to SANTE/11813/2017 (European Commission, 2017). The rice sample collected from an organic source was used as matrix for method validation. The extract obtained on extractions of the matrix was injected to LC- MS/MS and found free of tricyclazole (Fig. 2a). The sensitivity or linearity of tricyclazole with pure solvent as well as matrix matched calibrations showed that the method was linear with R^2 value of 0.999 with $\leq$ 20 per cent deviation of back calculated concentration from true concentration (Table 2). The matrix effect was found 15.06 per cent (Table 2). The detection and quantification limits for the tricyclazole were 0.0005 and 0.002 µg g^{-1}, respectively (Figs. 2b and 2c), which were far below the maximum residue level (MRL) (FSSAI, 2018). The per cent RSD between the response in reagent blank and blank control samples was <15 per cent and it showed less interference of rice matrix and also specific behaviour of tricyclazole. The trueness was evaluated and the mean recovery was found to be 91.21 to 99.75 per cent with relative standard deviation of <10 per cent (Table 3). For pesticide residues, main recovery values should be within the range of 70-120 per cent at each spiked level. The precision and robustness were evaluated and found as: repeatability (%) RSD of 5.454 to 7.133 for each spiked level i.e. 0.002, 0.01 and 0.02 µg g^{-1} (Table 4). The per cent RSD with respect to changed date of analysis was found < 10 per cent (Table 4).

The measurement uncertainty was based on a combination of top down and bottom up methodologies described in Eurachem guide (Eurachem, 2012). The sources of uncertainty for the method were the mass measurement of the standards for the preparation of solutions, detection of the standard solution, measurement of the volume of the extract, the recovery percentage and precision. Uncertainties related to measurements of volume and mass are negligible compared to other sources. For tricyclazole, the main contribution to the total uncertainty arose from the recovery percentage because this encompassed all the steps from the weighing of standards for preparation of solutions till the final quantification, including the whole extraction process, the instrument analysis and statistical processing of the data. The expanded uncertainty was determined at each fortification level and was in the range of 0.812 to 9.830. The overall result revealed that the method was efficacious, reliable and sensitive, enabling determination of tricyclazole residues in rice that may be present at trace level.

Table 3. Recovery of tricyclazole in rice at three different spiking levels

Replications	Recovery (%)		
	0.002 µg kg^{-1}	0.01 µg kg^{-1}	0.02 µg kg^{-1}
R1	100.90	94.90	93.70
R2	92.92	106.18	91.10
R3	93.86	99.20	91.64
R4	105.4	101.24	89.31
R5	91.44	96.38	85.23
R6	88.19	100.65	96.30
Mean	95.45	99.76	91.21
SD	6.43	3.99	3.79
RSD (%)	6.73	4.00	4.15

SD- Standard deviation; RSD = Relative standard deviation

Sample analysis

The validated method was applied to detect and quantify residues of tricyclazole in 51 rice samples acquired from different retailers. The residues of tricyclazole were not detected in 27 samples whereas in 23 samples these were at the LOQ or above (0.002-0.287 µg g^{-1}). Among the

Table 2. Linearity, limit of detection, limit of quantification and matrix effect of tricyclazole in rice

Tricyclazole	Regression equation	R^2	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Matrix effect (%)
Solvent standard	Y=40472X+26947	0.999	0.5	1.5	-
Matrix match standard	Y=42383X-54953	0.999	0.5	2.0	15.06

LOD = Limit of detection; LOQ = Limit of quantifications

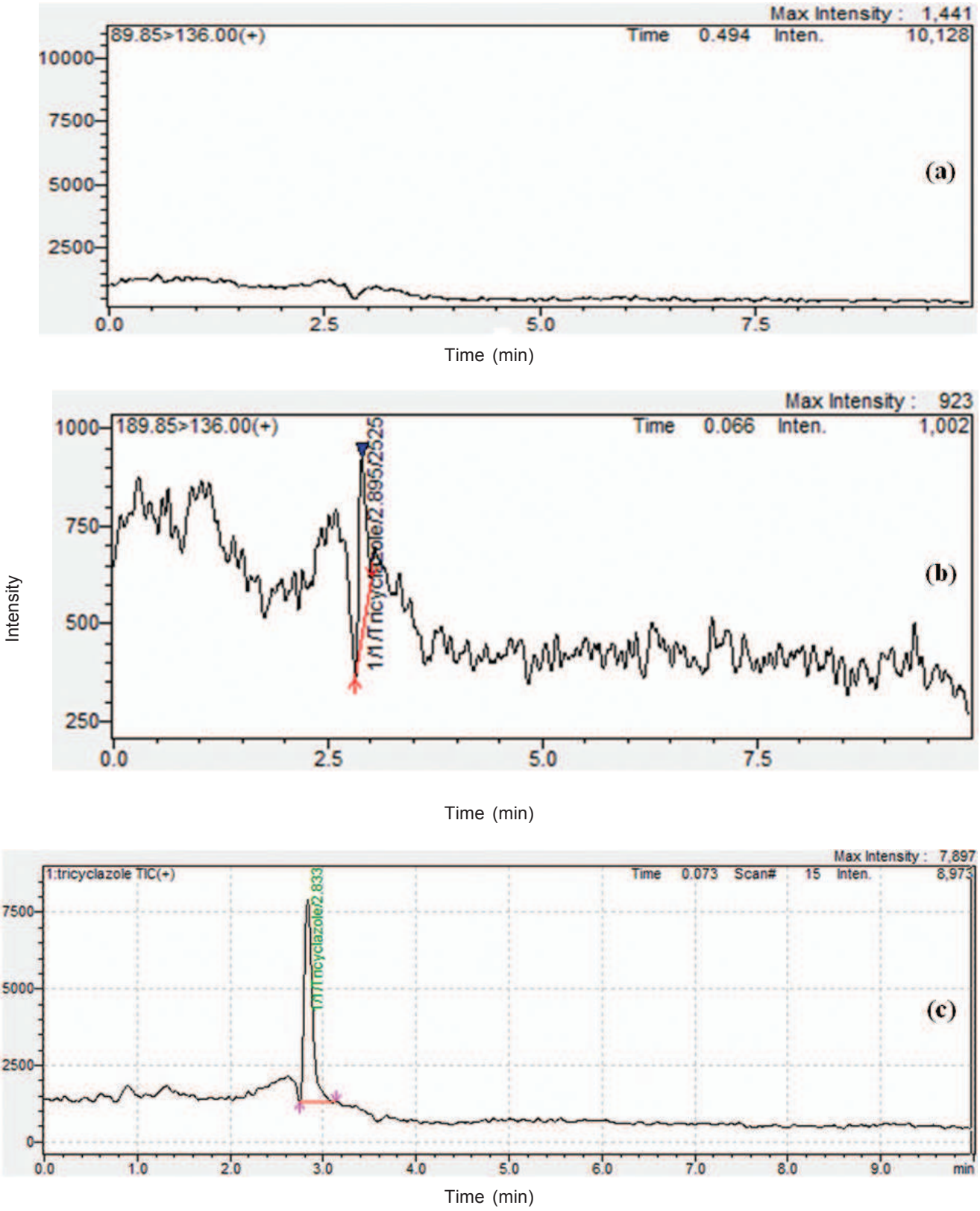

Figure 2: Chromatogram of tricyclazole in rice blank sample (a), rice matrix spiked at 0.0005 mg kg^{-1} (b) and rice matrix spiked at 0.002 mg kg^{-1} (c)

Table 4. Precision in terms of repeatability (RSD_r) and within-laboratory reproducibility (RSD_{wr})

Replication	RSD_r			$RSDw_r$					
	Recovery (%)			Recovery (%)					
	0.002 µg kg^{-1}	0.01 µg kg^{-1}	0.02 µg kg^{-1}	0.002 µg kg^{-1} 1st Day	0.002 µg kg^{-1} 2nd day	0.02 µg kg^{-1} 1st day	0.02 µg kg^{-1} 2nd day	0.01 µg kg^{-1} 1st day	0.01 µg kg^{-1} 2nd day
R1	94.98	104.77	87.13	101.01	101.03	96.89	100.33	99.80	93.13
R2	103.82	94.99	98.72	106.63	84.99	88.68	99.73	98.93	91.72
R3	91.84	98.01	102.43	98.42	97.32	84.66	98.46	101.04	88.43
R4	96.34	108.11	105.89	103.44	94.12	94.03	98.00	102.11	85.89
R5	92.6	96.19	96.57	90.59	90.29	84.19	97.77	103.19	97.57
R6	86.28	105.46	91.48	84.13	105.53	89.19	96.49	91.2	100.48
Mean	94.31	101.25	97.03	97.37	95.54	89.60	98.46	99.3	92.87
				93.48*	97.46*	95.66*			
SD	5.80	5.52	6.92	5.49	2.71	3.95			
RSD_r	6.15[(a)]	5.45[(a)]	7.13[(a)]	5.87[(b)]	2.78[(b)]	4.13[(b)]			

*Mean of between days, b= RSD_r, a= RSDwr, SD= Standard deviation

23 positive samples, 17 were of normal rice and 6 Basmati rice with the respective tricyclazole concentrations in the range of 0.002 to 0.287 µg g^{-1} and 0.007 to 0.167 µg g^{-1}. The tricyclazole residues in 16 samples (4 Basmati and 12 normal rice) were found to be above MRL fixed by FSSAI for tricyclazole in rice (FSSAI, 2018). Detection of tricyclazole residue in market rice samples could be attributed to its extensive application against the rice blast disease management. However, the relatively high concentration of tricyclazole in some of the samples is a matter of concern but assessment of risk associated with consumer health showed that there were no toxic effects of this chemical contamination in rice grains.

Health risk assessment

The calculated EDI values of tricyclazole residues were in the range of 0.000010 to 0.000961 and the HI values were less than one with a range of 0.002513 to 0.240363 in positive tested rice samples. It can be concluded that there is no apparent risk to the health of consumers of rice since the detected tricyclazole residues showed hazard index value below one. Hence, it is presumed that there are no acute effects on consumers from consumption of rice at the current level of contamination in north-eastern part of Karnataka, India. However, considering the possible accumulative effects of tricyclazole, precautionary measures should always be taken in the foreseeable future to safeguard consumer's health.

CONCLUSIONS

A simple, robust, QuEChERS method combined with LC-MS/MS for the determination of tricyclazole in rice has been reported. The duly validated method has considerable superiorities in respect of sample extraction, and the short time of analysis. Satisfactory LOQ, precision and accuracy were obtained, demonstrating the suitability of the method for tricyclazole residue analysis in rice for regulatory as well as routine residue monitoring purposes. Toxicological risk associated with consumption of rice was found to be nil. However, precautionary measures are suggested.

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