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Determination of 72 Chemical Pesticides and Estimation of Measurement of Uncertainty in Rice Using LC-MS/MS and GC-MS/MS

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Abstract

A multi-residue method was developed and validated for the simultaneous determination of 39 and 33 chemical pesticides in rice using LC-MS/MS and GC-MS/MS, respectively. The coefficient of determination was 0.994 to 0.999 over the linear concentration range of 0.01 to 0.1 μg mL^{-1}. The LOD and LOQ were in the range of 0.31 to 3.35 and 1.04 to 10.06 μgkg^{-1}, repsectively. The mean recoveries at 0.01, 0.05, and 0.1 mgkg^{-1} were found within the range 70 to 120% with satisfactory precision (RSD < 15%). Measurement uncertainty (U_{exp}) values estimated at 0.05 mgkg^{-1} spiking level were found lower than 20 μgkg^{-1} for all tested pesticides. A rice and wheat powder check samples under the proficiency test were analyzed using a standardized method, and the *Z* score recorded within the acceptable range of −2 to +2. The proposed method was applied to analyze the pesticides in real samples collected from the market and quantified the residues. The developed method could be useful for monitoring 72 chemical pesticides in rice matrices and can be adopted for the pesticide monitoring program.

Keywords Rice · LC-MS/MS · GC-MS/MS · Multi residue · Measurement uncertainty · Proficiency testing

Introduction

Rice, wheat, and corn are major cereal food grains. Among them, rice is the most consumed cereal product and a staple food for more than 70% of the world population (Pareja et al. 2011). More than 90% of the world's rice production is from Asia (IRRI 2006). It is a major grain cereal grown in over 100 countries and consumed by over two billion people in the world (Nguyen et al. 2008; Tsochatzis et al. 2010). Significant yield reduction due to insect pests and diseases made this crop to be a pesticide-intensive and being applied directly to the soil and on standing plants (Nguyen et al. 2008; Pengyan et al. 2006). The rice ecosystem provides constant niches for pest multiplication. Insect pests' damage in terms of yield loss is ranging from 21.0 to 51.0% and a significant reason for the continuous use of chemical pesticides in rice cultivation (Savary et al. 2012). The insect pest causes 33% total production loss (DWR Vision 2050 2015), and yellow stem borer, rice gall midge, delphacid planthoppers are key insect contributors to the loss (Savary et al. 2012). The weeds and disease infestation also contribute to a significant loss in grain yield and quality of rice. Rice accounts for 29.3% of the total pesticide preparations registered, followed by cotton, wheat, citrus, apple, corn, cabbage, cucumber, and cruciferous vegetables (Gu 2017). More than 50 different chemical pesticides belonging to organophosphate, synthetic pyrethroids, neonicotinoids triazole fungicides, and herbicides have been registered for use in rice cultivation (CIBRC 2018). The pesticide contamination needs to be monitored for their compliance with the MRLs set by the Food Safety Standard Authority of India, the Codex Alimentarius Commission, and the European Union. The maximum residue limit for pesticides was set in the range of 0.01 to 2.50 mgkg^{-1} by FSSAI and 0.01 to 1.00 mg kg^{-1} by the European Union for rice (FSSAI 2018; European Commission 2008).

To achieve the detection level below the MRLs for multi-class pesticides in rice commodity, a high sensitive multi-

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residue analytical methods involving LC-MS/MS and GC-MS/MS are most essential to screen more than 100 pesticides in a short time with few simple extraction techniques. The reviews on earlier research indicated that the use of solvents such as acetone in combination with dichloromethane, ethyl acetate, and acetonitrile for extraction of pesticide residue in food (Mastovska et al. 2004; Anastassiades et al. 2003) and separation of a wide range of polar pesticides was achieved through the development of multi-residue methods (Hercegová et al. 2007). The analysis of pesticides in rice and its various other products is of paramount importance. A simple, safe, cheap, high sample throughput method, namely QuEChERS in pesticide residue analysis, was developed and used around the globe with few modifications for contaminant analysis in food and other matrices (Anastassiades et al. 2003). Analysis of multi-class pesticide residues in different food matrices was achieved using a globally accepted extraction and clean-up technique *viz.*, QuEChERS technique, which found superior concerning accuracy (Anastassiades et al. 2003; Lehotay et al. 2005; Mastovska et al. 2004).

The pesticide residues have been reported in in wheat, wheat flour, and bran (Kolberg et al. 2011); thiamethoxam residues in brown rice and oats, and imidacloprid in maize (Wang et al. 2012) was reported. The review of multi-class pesticide residue analysis revealed the presence of alpha-endosulfan (0.1 $\mu g g^{-1}$), deltamethrin (0.067 $\mu g g^{-1}$), and fipronil (0.01 $\mu g g^{-1}$) (Uddin et al. 2011; Tomer 2013); chlorpyrifos in rice samples (Zhang et al. 2012). The quantification of multi-class pesticide residues in different food commodities is essential to analyze more samples in a short analysis time. The screening of 200 pesticides in rice, corn, and wheat flour with LOQ is in the range of 5–50 $\mu g\ kg^{-1}$ (He et al. 2015); 122 pesticides in wheat, barn, and corn using GC-MS/MS (Walorczyk 2007); 300 pesticides using GC-EI-MS/MS employing QuEChERS method of extraction and clean up were the reported methods and provides compliance with meeting requirements for EU MRLs (Hernandez et al. 2013; Banerjee et al. 2012).

The review of analytical methods being suitable for analysis of multi-class pesticides in rice and other cereal matrices were reported. The process for analysis of phenoxy-acid herbicides, phenyl urea herbicides was reported (Koesukwiwat et al. 2008; Mol et al. 2008); quantification of 22 pesticides in white and wild rice (Aguilera et al. 2005); 47 pesticides in cooked and polished rice (Lee et al. 2009) was developed and validated. Method for analysis of more than 90 pesticides in rice matrix was developed and validated (Anastassiades et al. 2003; Libin et al. 2006; Liu et al. 2007; Takatori et al. 2008). Furthermore, a tandem mass spectrometry-based method for simultaneous determination of 109, 185, 203, and 405 pesticides in unpolished and polished rice was reported (Zhang et al. 2011; Mastovska et al. 2009; Nguyen et al. 2008; Pengyan et al. 2006) and multi-residue analysis of over 200 pesticides in cereals (He et al. 2015); analysis of 310 pesticide multi-residues in brown rice, orange, and spinach (Lee et al. 2018); in rice and wheat flour (Grande-Martínez et al. 2016) and analysis of four sulfonylurea herbicides in cereals (Ni et al. 2018) are some of the recently published methods. Several of these methods lacked assessment of uncertainty measurement with the technique and at various levels of sample preparation protocol in contaminant analysis. The measurement uncertainty is the quality attribute of a measurement result. The uncertainty value must be such that the measurement results are fit for the intended purpose. This ensures that risks associated with compliance decisions are within acceptable limits. Therefore, correct estimation of measurement result and its associated uncertainty is essential for any method and sample preparation protocol being developed for trace-level analysis (NABL document 141 2016).

Pesticide usage has created a massive concern regarding food quality. MRLs are set to protect the consumers and are based on authorized uses of plant protection products. Several international and referral institutions have developed food standards for checking the quality of rice and other food materials. The Food Safety Standard Authority of India for enforcement MRLs of pesticide residues for indigenous and export rice in India. European Union (EU) has established the maximum residue level (MRL) in rice grains under EU Regulation (EC) No. 396/2005 (European Community Regulation, 396/2005). The multi-residue analytical method should fulfill the regulatory agency requirements and must have acceptable estimated uncertainty values for all the tested analytes, which can make the samples results free from false positive and negative results. The developed method should be adaptable for the analysis of pesticides in the check samples, farmer's field, rice processing units, and dealers, which helps know the pesticide usage pattern in various rice production systems. With these objectives, a method was developed and validated for the simultaneous determination of 72 chemical pesticides in rice matrix and estimated uncertainty measurement. The developed method was used to screen the check samples provided under the proficiency test and also real samples for its efficiency.

Materials and Method

Chemicals, Reagents, and Instruments

Certified reference materials having a purity of more than 99.00% were obtained from Dr. Ehrenstorfer (Augsburg, Germany), LC-MS grade acetonitrile and methanol (purity ≥ 99.90%) were procured from J. T. Baker (NJ, USA), ethyl acetate (purity ≥ 99.80%) was purchased from Merck (Mumbai, India). Ammonium formate and formic acid (≥ 90.00% purity) were purchased from Empart,

Hyderabad, India. Anhydrous magnesium sulfate, sodium acetate, and sodium sulfate (>99.00% purity) were procured from Himedia, Bangalore, India. Solid-phase extraction sorbent; primary, secondary amine (PSA) (AR grade, the particle size of 40μm) was obtained from Agilent Technologies India Pvt. Ltd. (Bangalore, India). HPLC grade water was obtained with a Milli-Q water system (Millipore, Billerica, MA, USA).

GC-MS/MS (Shimadzu, GCMS TQ 8030®) and LC-MS/MS (Shimadzu, LC-MS 8040®) were used for method development, qualitative and quantitative analysis of pesticides. Analytical balance (Make-Sartorius® and sensitivity range-6 digits), centrifuge tube (Tarsons®), homogenizer (IKA®-T_{18}), low and high volume centrifuges (Gyrozen® -1736R and 2236R), vortexes (REMI®), turbovap-LV evaporator (TurboVap®), horizontal shaker (Rotek®) were employed for the extraction of pesticide residues from different samples used in this study.

Blank Matrix and Pesticide Selection

The pesticide-free rice grains polished rice, and wheat powder samples were collected from the organic products outlets at local markets, Raichur, India. Paddy sample was obtained from an organically cultivated field and bran of the paddy samples utilized for conducting recovery experiments. These matrix blanks were checked for the presence of pesticides and used further in the method validation process. A total of 72 pesticides were selected for the study includes organochlorine, cyclodeins, organophosphates, carbamates, synthetic pyrethroids, insect growth regulators, new chemistry group insecticides, and different group fungicides and herbicides.

Preparation of Standard Stock Solution

The standard stock solution of individual pesticide was prepared by accurately weighing 10 to 12 ± 0.01 mg of each CRMs, transferred into a 10 mL clean, calibrated volumetric flask, and dissolved using 10 ml of methanol and ethyl acetate-based on suitability with LC and GC techniques, respectively, resulting in a final concentration of approximately 1000 μg mL^{-1}. An intermediate and working standard solution of 100 and 10 μg mL^{-1}concentration was prepared by the serial dilution method. The mixture of 38 LC and 34 GC compatible pesticides at 0.5 μg mL^{-1} concentration was prepared from 100 μg mL^{-1} stock by drawing 50 μL of each standard in 10 mL volumetric flask and volume made up with methanol and ethyl acetate separately. A serial dilution technique was used to prepare working standard mixture of 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1μgmL^{-1}. All solutions such as stock, intermediate, and the working solution were stored at −20°C. The matrix match standards at the same concentrations were prepared by using the blank rice matrix extract.

Sample Extraction and Clean-up

The QuEChERS method originally published by Anastassiades et al. (2003) and Lehotay et al. (2005) for extraction and determination of pesticide residues in produce was followed with modification explained below for extraction of pesticide residues in rice and validated.

Weighed accurately 5 g of homogenized rice sample using an analytical balance and transferred into a 50-mL centrifuge tube and then 10 mL of chilled distilled water was added and kept for 10 min. at room temperature. Later, 10 mL of 1% ethyl acetate in acetonitrile was added, and the mixture was shaken vigorously for 30 seconds. Subsequently, for dispersive solid-phase extraction, 6 g of anhydrous magnesium sulfate and 1.5 g sodium acetate was added and vortexed for 1 min. and then homogenized at 10000-13000 rpm for 3 min. The content was centrifuged at 5000 rpm for 5 min. and 7 mL of supernatant was transferred into a 15-mL centrifuge tube containing 350 mg PSA and 1.05 g anhydrous magnesium sulfate and then vortexed for one minute. The sample was again centrifuged at 12000 rpm for 5 min. Then, transferred 3 mL of each extract (separately for GC-MS/MS and LC-MS/MS test) into the test tubes containing 300 μL of 10% DEG (Diethylene glycol) evaporated the content using a nitrogen flash evaporator at 35 °C to dryness. Later reconstituted the residue with 1.5 mL of methanol and 1.5 mL of ethyl acetate, and the mixture was sonicated using an ultrasonicator to dissolve residues completely. Then, the mixture was filtered through a 0.22-μ PTFE nylon syringe filter into a vial for LC-MS/MS and GC-MS/MS analysis separately.

LC-MS/MS Parameters

The LC-MS/MS system equipped with SHIMADZU 1200 series UHPLC and LCMS 8040 with triple quadrupole MS/MS detector was employed for analysis. The data acquisition and processing were done with LabSolution® 1.5 version software. C18 column (Shimpack XR, octadecylsilyl; ODS-III, 2 mm i.d x 150 mm) was used for chromatographic separation. LC conditions as follows: mobile phase (A) consisted of 5 mM ammonium formate, 2 ml methanol, and 0.01% formic acid and made-up to the volume of 100 mL using HPLC water (2:98 v/v methanol: water) and a mobile phase (B) consisted of 5mM ammonium formate, 0.01% formic acid and made up to the volume of 100 ml with 100% methanol. The column flow rate of 0.4 mL min^{-1}; 2 μL injection volume, and the column temperature was 40 °C. The mobile phase gradients program of the binary pump was 60% A and 40% B at the beginning for 15 min. followed by 100% B for 5 and then 60% A, 40% B for 5min. The mass spectrometer was operated in electrospray ionization (ESI+) mode, interface current was 0.1 μÅ, heat block temperature was 400 °C, DL temperature was 250 °C, nebulizer gas Nitrogen (2.9 L/min.),

drying gas of 15 L/min, CID gas: Argon (230 kpa). To achieve the highest sensitivity, the fragment, voltage, and the collision energy were optimized.

GC-MS/MS Parameters

The gas chromatograph (Shimadzu, GC 2100) equipped with MS triple quadrupole system (GC-MS TQ 8030) coupled with an electronic flow controller (EFC), an AOC- 20i injector, and AOC-20s autosampler was used in this study. Data acquisition and processing were performed with LabSolution® software. The injection volume was 2μl in splitless mode, surge pressure of 250 kpa, and injector temperature was 280 ^{o}C. A capillary fused silica HP 5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for the separation of the pesticides. The column oven temperature program as follows; initially, the oven temperature was 60 °C held for 2 min. 25 °C min^{-1} rate to 150 °C, the 3 °C min^{-1} rate to 200 °C and further, 8 °C min^{-1} rate to 280 °C held for 5 min. and finally, 25 °C min^{-1} rate to 300 °C for 3 min. holding time. All the pesticides separated clearly within the total run time of 41.07 min. The carrier gas was helium (99.999% purity), with a constant flow rate of 1.5 mL min^{-1} was maintained. MS parameters in the GC-MS/MS were as follows: transfer line temperature was 280 °C, and the ion source temperature was 250 °C was maintained. Ionization by the positive electron impact (EI) mode using electron energy of –70 eV. The MRM scan mode was selected. The solvent delay was fixed at 3 min. Argon (Ar) and helium (He) gases with flow rates of 1.50 and 2.25 mLmin^{-1} were used as collision and quench gases, respectively. The mass range was between 50 and 550 m/z.

Method Validation

The method was validated according to the SANTE/12682/2019 by evaluating different parameters: linearity, the limit of detection (LOD), the limit of quantification (LOQ), specificity, trueness (bias), precision in terms of repeatability (RSDr-intraday), precision in terms of reproducibility (RSDwR-interday) and matrix effect.

Linearity

The linearity of the method was assessed in both solvent and matrix-matched by injecting the standard concentrations for all the pesticides. A six-point calibration curve at 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 μgmL^{-1} was prepared from 0.5 μg mL^{-1} working standard mixture in methanol and ethyl acetate for LC and GC techniques, respectively.

LOD and LOQ

The LOD was calculated by preparing different solutions of pesticide mixture with a low concentration that is expected to produce a response in the LC-MS/MS and GC-MS/MS. LOQ is the lowest spike level meeting the identification and method performance criteria for recovery, and precision criteria must be less than or equal to the MRL of the particular pesticide. LOQ was identified by spiking the lowest concentration of pesticide to the blank matrix and extracted following the procedure explained above and injected into the instrument. The recovery was recorded within the limit of 70–120%, with an RSD of ≤ 20%. All the pesticide recorded LOQ less than 10 μgkg$^{-1,}$ which were below the MRL and compliance with the SANTE/12862/2019 guidelines. The LOD and LOQ were estimated following the guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in feed and food (Wenzl et al. 2016; Magnusson and Örnemark 2014).The mathematical formulae employed as follow

$$X_{LOD} = 5.2 \times S_{y,}net/b$$
$$X_{LOQ}=3.3 \times X_{LOD}$$

where X_{LOD}: limit of detection

$S_{y,}net$: standard deviation of the precision data at lowest spike level
b: slope of the calibration curve
X_{LOQ}: limit of quantification

Trueness

The recoveries of pesticides in rice were done by spiking to the rice matrix at 0.01, 0.05, and 0.1 μg/g with six replication and along with a non-spike blank sample as a control. The spiked samples at each level were then kept at room temperature for 25 °C for 30 min to attain sample stability. The fortified samples were extracted by following the extraction and clean-up procedure described above. The extracted aliquots were injected into LC-MS/MS, and GC-MSMS and the area under the peaks of the known amount of pesticides in the spiked matrix before the extraction and with sample extract spiked near the chromatographic analyses (matrix-matched standards) was compared to calculate percent recoveries.

Matrix Effect

The angular coefficients values obtained with solvent and matrix calibration curves were considered to calculate the matrix effect using the following equation,

$$\text{Matrix effect}\ (\%) = (\text{bm}-\text{bs})/\text{bs} \times 100$$

where bm and bs are the angular coefficient of the calibration curve in the matrix and solvent, respectively (European Commission 2019; Silva et al. 2012).

Precision (Intra- and Inter-day Assay)

The method intraday precision was ascertained regarding the repeatability relative standard deviation (RSDr) of the six replicates exactly similar extractions of blank samples spiked with pesticide at the concentration level of 0.05 μg g^{-1} and injected into LC-MS/MS and GC-MS/MS on the same day. The interday precision was estimated concerning reproducibility (RSD_wR) of six replicates by doing the fortification of standards at a concentration of 0.05 μg g^{-1} at two different dates, analysts with six replications (interday).

Mass Selection (Ion Ratio)

As per the SANTE/12862/2019, a minimum of 2 product ions (mass to charge ratio) with the ion ratio from sample extracts should be within ± 30% (relative) of the average of calibration standards from the same sequence required for detection, confirmation, and quantification. In this study, three m/z values, such as parent ions mass (one) and product ion mass (two) for each pesticide along with their corresponding collision energies (CEs) were selected (Table 1).

Calculation of Measurement of Uncertainty

The expanded uncertainty (U_{exp} at 95% confidence limit) was calculated by the addition of a coverage factor of 2 at a confidence level of 95% for a fortification level of 50 μg kg^{-1} as per the statistical method mentioned in EURACHEM/CITAC Guide (Ellison and Williams 2012; NABL document 141 2016; Banerjee et al. 2007; Kanrar et al. 2010; Naik et al. 2020).

Different sources of uncertainty were taken into account, such as Uncertainty due to repeatability (6 replicate) (U1), balance for weighing of the sample (U2) and weighing of standards (U3), 10-mL volumetric flask for preparation of 1000 μg mL^{-1} stock solution (U4), 10-mL volumetric flask for preparation of 100 μg mL^{-1} intermediate solution (U5), 10-mL volumetric flask for preparation of 10 μg mL^{-1} working solution (U6), 10-mL volumetric flask for preparation of 0.5 μg mL^{-1} working solution (U7), 100-μL micropipette for preparation of calibration curve (U8), calibration curve (U9), recovery of particular analyte (U10), and certified reference material (U11) were calculated. Furthermore, the combined uncertainty (U_c) was calculated by multiplying the mean value of repeatability result and summation of square of all individual uncertainty from U1 to U11. Coverage factor, k=2 at 95% confidence level, was considered for estimation of expanded uncertainty (U_{exp}). The equations used in the assessment of measurement uncertainty are as follows,

Uncertainty due to repeatability (Type 'A' Uncertainty),

$$U = \frac{\text{Standard Uncertainty}}{\text{Mean}}$$

Combined Uncertainty (Type 'B' Uncertainty),

$$U_c = \text{Mean value of repeatability result} \times \sqrt{\text{Summation of the square of all individual uncertainty from U1 to U11}}$$

Coverage factor (k) at 95% confidence level

$$V_{\text{effective}} = \frac{(U_C)^4}{\frac{(U_1)^4}{(d.f.)} + \frac{(U_2)^4}{(d.f.)} + \ldots + \frac{(U_n)^4}{(d.f.)}}$$

where

U_C= Combined Uncertainty
U_1, U_2, …U_n = Relative Uncertainty of individual component
d.f = degrees of freedom for an individual component

Expanded Uncertainty (at 95% confidence; k=2), $U_{exp} = U_c$ x k (Naik et al. 2020)

Proficiency Testing in Cereal Samples

The proficiency testing in pesticide residue analysis was attended for two different cereal matrices, such as rice and wheat powder. The sample extraction and clean-up for rice and the wheat powder were attended following the standardized method. The quantification of pesticides was attended using LC-MS/MS and GC-MS/MS. The proficiency was measured in terms of "Z" score upon the residue of pesticides against the actual concentration spiked. The calculation for "Z" score using following the mathematical formulae,

$$Z_i = \frac{(X_i - X_{pt})}{\sigma_{pt}}$$

where X_i = participants value; X_{pt} = assigned value; σ_{pt} = standard deviation for proficiency assessment. When there is a concern about the assigned value, i.e., u (x pt), > 0.3 σ_{pt}, the uncertainty of assigned value is taken into account while estimating the performance score, then modified Z score (Z' score) was calculated using formulae,

Table 1 Retention time, mass spectrometric parameters for multiclass pesticides

Pesticide	RT (Min.)	Quantification		Confirmation			
		MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)
GC-MS/MS							
Phenol, 4-bromo-2-chloromo	6.56	208.0>63.10	27	208.0>99.10	21	208.0>144.00	15
Trifluralin	12.34	110.1>64.0 18	18	152.1>110.1	8	110.1>92.0	12
Alpha-BHC	12.81	306.1>264.1	8	306.1>206.1	14	264.1>206.1	8
Beta-BHC	14.04	218.9>182.9	8	180.9>144.9	16	180.9>74.0	30
Diazinon	15.17	304.1>179.1	10	304.1>162.1	8	304.1>137.1	26
Fluchloralin	15.38	306.0>264.10	6	306.0>160.20	27	306.0>206.20	18
Tri-allate	15.67	268.1>226.0	14	268.1>184.0	20	270.1>228.0	14
Iprobenfos	16.06	204.0>91.0	8	204.0>171.0	6	123.0>45.0	16
Chlorpyrifos-methyl	17.31	285.9>93.0	22	287.9>272.9	20	287.9>93.0	22
Parathion-methyl	17.30	125.0>47.0	12	263.0>109.0	14	125.0>62.0	6
Alachlor	17.73	188.1>160.1	10	188.1>132.1	18	160.1>132.1	10
Heptachlor	17.50	271.8>236.9	20	271.8>117.0	32	271.8>201.9	38
Fenitrothion	18.77	277.0>260.0	6	277.0>109.1	14	260.0>125.1	22
Aldrin	19.20	292.9>219.9	26	292.9>257.9	16	292.9>186.0	40
Chlorpyrifos	19.91	313.9>257.9	14	313.9>285.9	8	313.9>193.9	28
Parathion	19.95	291.1>109.0	14	291.1>137.0	6	291.1>81.0	24
Chlorfenvinphos (E)	22.21	267.0>159.0	18	323.0>267.0	16	323.0>295.0	6
Phenthoate	22.34	119.1>82.1	28	119.1>84.1	28	149.1>105.1	4
Butachlor	23.76	188.1>160.1	12	188.1>132.1	18	176.1>134.1	12
p,p'-DDE	24.53	246.0>176.0	30	246.0>211.0	22	317.9>248.0	24
Endrin	25.22	262.9>193.0	28	262.9>228.0	22	244.9>210.0	8
Beta-Endosulfan	25.62	338.9>160.0	18	338.9>266.9	8	338.9>195.9	20
p,p'-DDT	26.12	235.0>165.0	24	235.0>199.0	16	237.0>199.0	16
Endosulfan sulfate	27.13	386.8>288.8	10	386.8>252.9	16	386.8>240.9	22
o,p'-DDT	27.34	235.0>165.0	24	235.0>199.0	16	237.0>199.0	16
Bifenthrin	29.08	181.1>166.1	12	181.1>153.1	8	181.1>179.1	12
Fenpropathrin	29.23	265.1>210.1	12	265.1>89.0	28	181.1>127.1	28
Lambda-Cyhalothrin	30.55	181.1>152.1	24	163.1>127.0	14	-	-
Permethrin I & II	31.49	163.1>127.1	8	183.1>165.1	14	183.1>153.1	14
Cyfluthrin (4 peaks)	32.56	226.1>206.1	14	226.1>199.1	6	163.1>91.0	14
Etofenprox	33.16	163.1>135.1	10	163.1>107.1	18	163.1>95.0	18
Fenvalerate	34.20	419.1>225.1	6	419.1>167.1	12	419.1>125.1	26
Deltamethrin	35.64	252.9>93.0	20	252.9>171.9	8	252.9>77.0	26
LC-MS/MS							
Acephate	1.06	183.9>142.95	11	183.9>49	23	183.9> 95.05	26
Omethoate	1.09	214>125	24	214>182.95	12	214>109.05	30
Pymetrozine	1.32	217.9>105.1	22	217.9>78	44	217.9>51.15	54
Imidacloprid	2.03	256.05>209.1	17	256.05>221.15	9	256.05>175.05	19
Dimethoate	2.67	229.95>199	10	229.95>125	22	229.95>171	17
Carbendazim	3.05	191.95>160	18	191.95>132	32	191.95>105.05	40
Thiacloprid	3.22	253.2>126.05	23	253.2>99.1	48	253.2>90.05	40
Phosphomidon	4.65	299.9>174.1	14	299.9>127.05	28	299.9>132.1	24
Bendiocarb	5.42	224>167.1	11	224>109	19	224>81.1	35
Metribuzin	5.43	215>187.1	20	215>49	29	215>58.05	27
Carbofuron	5.47	221.8>165.1	12	221.8>123.1	23	221.8>55.05	29

Table 1 (continued)

Pesticide	RT (Min.)	Quantification		Confirmation			
		MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)
Carbaryl	6.12	202>145.05	12	202>127	28	202>117.1	24
Isoproturon	7.48	206.9>72.1	23	206.9>46.15	18	206.9>165.1	15
Metalaxyl	7.52	279.8>220.2	15	279.8>192.15	18	279.8>248.15	11
Chlorantraniliprole	8.31	483.75>452.95	17	483.75>285.9	16	483.75>177.05	50
Coumatetryl	9.11	293.1>175.1	24	293.1>91.15	36	293.1>107.05	35
Paclobutrazole	9.47	294.25>70.15	23	294.25>125.1	37	294.25>43.15	50
Methoxyfenozide	9.63	368.95>149.05	18	368.95>313.15	9	368.95>133.05	24
Triademefon	9.72	293.9>69.1	23	293.9>197.1	17	293.9>225.1	14
Triazophos	9.98	313.65>162.1	19	313.65>119.1	35	313.65>97.05	37
Triademenol	10.04	296.25>70.05	12	296.25>43.15	47	296.25>99.1	18
Tetraconazole	10.4	371.8>159.05	34	371.8>70.15	25	371.8>150.1	35
Metalachlor	10.64	283.95>252.15	15	283.95>176.2	27	283.95>134.1	34
Quinalphos	11.2	299.2>163.15	22	299.2>147.1	22	299.2>119	44
Penconazole	11.37	284.25>70.15	18	284.25>159.05	31	284.25>267.2	8
Benalaxyl	11.55	325.8>148.15	23	325.8>294.15	12	325.8>208.1	16
Hexaconazole	11.86	314.1>70.15	22	314.1>297.2	10	314.1>45.1	39
Bitertenol	12.03	338.2>269.2	10	338.2>99.1	17	338.2>70.15	11
Phosalone	12.05	367.7>182.05	16	367.7>111.05	43	367.7>138.1	32
Spinosad	12.17	732.2>142.15	35	732.2>98.25	54	732.2>99.15	52
Thiobencarb	12.25	258.05>125.05	20	258.05>100.15	13	258.05>89.15	49
Difenconazole	12.46	406.05>251	28	406.05>111.05	55	406.05>188.05	47
Pretilachlor	12.81	311.9>252.15	16	311.9>176.2	29	311.9>147.1	40
Profenofos	13.13	374.95>304.9	21	374.95>346.95	14	374.95>128.15	47
Emamectin benzoate	13.31	886.3>158.15	42	886.3>126.15	48	886.3>82.2	50
Buprofezin	13.51	306.15>200.9	11	306.15>57.15	30	306.15>116.1	16
Hexythiazox	14.00	352.9>228	15	352.9>168.15	28	352.9>116.15	43
Pendimethalin	14.18	281.8>212.05	11	281.8>199.95	8	281.8>193.95	20
Fenpyroximate	14.62	422>366.2	16	422>138.2	35	422>215.15	27

$$Z'_I = \frac{(X_i - X_{pt})}{\sqrt{\sigma^2_{pt} + u^2\,(x_{pt})}}$$

where X_i = participants value, X_{pt} = assigned value, σ_{pt} = standard deviation for proficiency assessment, and $u(X_{pt})$ = standard uncertainty of assigned value (NABL document 162 2008; Naik et al. 2020). Whereas the uncertainty of the assigned value is calculated as

$$u(X_{pt}) = 1.25 \times S'/\sqrt{p}$$

where u_x is the uncertainty in mgkg^{-1}; S' is the robust standard deviation estimate; and p is the total number of participants results.

Suitability of the Method

The method validation guidelines clearly stated that, for analysis of pesticide residues in cereal grain matrices and products thereof, the method validation must be performed in any one of the cereal matrices and validation hold good for all other matrices of similar nature in their composition of high starch and/or protein content and low water and fat content (European Commission 2019). The developed and validated method in rice matrix was employed to confirm its suitability for other cereal matrices such as polished rice, wheat flour, paddy grain, and rice bran. The suitability of the standardized method was evaluated in terms of recovery/accuracy at spiking of pesticide mixture concentration of 0.05 mg kg^{-1}.

Monitoring of Pesticide Residues

A total of 100 different paddy and 50 rice samples were collected from farmers' field and rice processing industries, respectively. Extraction and clean-up of the samples were made as per the procedure given in the previous section. The samples were screened with the developed method, and pesticides were quantified.

Results and Discussion

Optimization of LC-MS/MS

The chromatographic separation with the optimized parameters in LC-MS/MS resulted in better peak resolution for all the tested pesticides. Full scan mass spectrums of all the pesticides were recorded to select the polarity and most abundant m/z ion (mass-to-charge). The protonated $(M+H)^+$ molecular ion in case of positive polarity or deprotonated molecular ion $(M+H)^-$ in case of negative polarity was determined and chosen as the precursor ion. Further optimization of collision energy and voltages was done automatically with the auto-optimization option in the LC-MS/MS software (Labsolution®) and multiple reaction monitoring (MRM) transitions were created for all the pesticide (Table 1). The multiple reaction monitoring (MRM) transitions and associated acquisition parameters were optimized with electrospray ionization (ESI positive mode) in the tandem mass spectrometer. The optimized precursor and product ion transitions with collision energy (ECs) were used for identification, quantification, and confirmation of different pesticide residue in real samples. The developed MRM (multiple reaction monitoring) modes provide high sensitivity and selectivity required for an analytical method to detect the multi-class pesticides in rice matrix at a concentration of 0.01 mg kg^{-1} (Table 1). The selection of a minimum of three different ions (for quantification and confirmation of analytes) for all the pesticide tested in this study fulfilled the requirements as per the SANTE/12682/2019 (European Commission 2019).

Optimization of GC-MS/MS

GC-MS/MS optimization was done by injecting the multiclass pesticide standard mixtures (1.00 mgkg^{-1}) and analyzed using scan mode. Individual pesticide retention times were identified and precursor ions were selected. The precursor ion as a base ion in the mass spectrum showed the highest intensity and product ions selected for confirmation of the pesticide in the matrix. Furthermore, product ions scan was performed with different Collision Energies (CE) to ensure the specificity and sensitivity in the determination of pesticides with this method. For each pesticide, multiple reaction monitoring (MRM) transitions with appropriate CEs were determined (Table 1) and further simplified by using the smart MRM optimization tool. GC-MS/MS MRM mode offers high specificity and simultaneous determination of pesticide compounds in the rice matrix. Initially, three to four transitions were selected for each pesticide to determine the most suitable m/z ion (mass-to-charge) for quantification and qualification and which ensured the specificity and sensitivity of the method. These MRM transitions were then registered to Smart Database containing the information on different transitions of the pesticides. The optimum time segments and three MRM transitions per compound were generated. The ion ratio between the intensity of the quantifier and qualifier ions was used as a confirmatory parameter of pesticides analyzed in rice matrices and the ion ratio was found to be within the acceptable limit of ± 30% at all spiked concentrations (European commission 2019).

The optimization of LC-MS/MS and GC-MS/MS parameters found in accordance with previous studies on multi-group chemical pesticides using gas chromatography tandem mass spectrometry and liquid chromatography tandem mass spectrometry. Method optimized by Anastassiades et al. (2003) using GC-MS; Libin et al. (2006) using GC-MS to quantify 97 pesticides in 90 minutes; Liu et al. (2007) using GC-MS with gel peramiation chromatography; Takatori et al. (2008) using LC-MS/MS for multiresidue method in fruits and vegetables; Mastovska et al. (2009) using GC–TOFMS and UPLC–MS/MS in rice for multi pesticide residues; Nguyen et al. (2008) using GC-MS/MS to quantify 203 pesticide in rice and Pengyan et al. (2006) using GC-MS/MS found very sensitive and reproducible analytical method for simultaneous determination of multiple pesticides.

Validation of the Method

The method validation parameters are summarized in Table 2 for LC and GC compatible pesticides. The calibration curve for each pesticide was linear over the concentration ranged from 0.01 to 0.1 μg $mL^{-1,}$ and the coefficient of determination (R^2) ranged from 0.994 to 0.999. The LOD and LOQ were in the range of 0.31 to 3.35 and 1.04 to 10.06 μgkg^{-1}, repsectively. The LOD and LOQ values of tested pesticides in the present validated method were below the corresponding MRLs for rice grains. The LC-MS/MS and GC-MS/MS techniques were highly sensitive for tested pesticides and provide compliance to the Maximum Residual Limit (MRLs) established by the Food Safety Standard Authority of India and the European Union (Table 2).

The rice (prepared) sample was fortified ($r = 6$) with mixed pesticides standard solution at the concentration of 0.01, 0.05, and 0.1 mgkg^{-1}. The recoveries at all the spiking levels were within the range of 70.00 to 120.00% for the 72 tested pesticides with a relative standard deviation of less than 20%

Table 2 Linearity range, regression equation, recovery, and RSD of 72 pesticides in rice matrix

Pesticide	Linear range (μgkg^{-1})	Regression equation	R^2	LOD (μgkg^{-1})	LOQ (μgkg^{-1})	Recovery (%) and RSD					
						0.01 mg kg^{-1}		0.05 mg kg^{-1}		0.10 mg kg^{-1}	
						Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Phenol, 4-bromo-2-chloromo	10-100	Y=919.1212X + 1445.618	0.998	3.09	9.35	77.16	12.43	79.95	2.58	89.12	3.73
Trifluralin	10-100	Y=1032.891X - 1977.603	0.998	3.32	9.98	77.74	4.30	88.46	2.99	93.73	3.49
Alpha-BHC	10-100	Y= 968.6393X - 819.7868	0.999	3.35	10.05	70.82	7.32	87.78	1.24	90.39	1.58
Beta-BHC	10-100	Y=743.95X - 712.7537	0.999	2.04	6.17	72.08	6.78	89.75	3.53	92.34	1.20
Diazinon	10-100	Y=539.5818X - 1170.41	0.997	3.34	10.03	78.60	12.06	85.65	4.55	89.81	2.91
Fluchloralin	10-100	Y=438.2034X - 1519.544	0.995	3.25	9.76	77.64	15.13	86.03	7.83	89.31	4.16
Tri-allate	10-100	Y=463.6644X - 1345.646	0.995	3.04	9.21	70.23	12.87	86.12	2.99	84.70	6.93
Iprobenfos	10-100	Y=2589.383X - 1305.828	0.999	3.28	9.93	77.82	6.69	86.92	2.27	91.65	2.04
Chlorpyrifos-methyl	10-100	Y=677.3839X - 922.6327	0.999	3.29	9.88	73.82	5.53	86.63	2.29	90.05	3.88
Parathion-methyl	10-100	Y=760.426X - 2073.986	0.996	2.86	8.65	75.69	8.79	84.49	5.82	90.23	2.45
Heptachlor	10-100	Y=862.6217X - 804.6414	0.994	3.34	10.03	72.14	9.18	85.79	3.71	94.74	3.45
Alachlor	10-100	Y= 1485.377X - 1957.071	0.999	2.80	8.49	78.42	8.03	87.39	3.57	93.29	2.10
Fenitrothion	10-100	Y=682.1321X - 1845.936	0.994	3.35	10.05	71.07	13.52	83.37	7.59	90.79	2.13
Aldrin	10-100	Y=255.0467X - 334.2646	0.994	3.18	9.59	77.12	16.62	87.09	7.16	90.97	3.73
Chlorpyrifos	10-100	Y= 710.5104X + 148.5997	0.999	3.27	9.82	92.50	3.72	92.82	2.31	97.60	4.05
Parathion	10-100	Y=388.5953X - 1462.617	0.992	3.34	10.03	84.61	12.93	82.52	8.20	91.23	5.26
Chlorfenvinphos (E)	10-100	Y=938.7536X - 696.9756	0.997	2.19	6.59	71.83	8.02	85.82	2.06	91.43	2.65
Phenthoate	10-100	Y=915.1492X - 1217.772	0.997	1.98	5.95	71.43	6.46	87.58	1.87	87.85	2.83
Butachlor	10-100	Y= 883.536X + 3761.909	0.999	3.29	9.87	110.95	3.91	101.32	3.44	112.75	2.17
p,p'-DDE	10-100	Y=1970.774X + 87.9295	0.999	3.34	10.04	83.91	2.08	93.22	1.55	93.81	0.90
Endrin	10-100	Y=156.2297X - 444.3429	0.998	3.01	9.12	78.02	12.06	80.56	9.44	95.63	2.94
Beta-Endosulfan	10-100	Y= 32.4718X - 322.0507	0.994	3.35	10.05	79.43	1.67	72.21	11.21	78.33	10.87
p,p'-DDT	10-100	Y=6893.367X - 1459.905	0.999	1.60	4.84	91.92	3.28	91.70	1.14	92.92	2.13
Endosulfan sulfate	10-100	Y= 51.23937X - 296.8668	0.998	3.35	10.06	78.00	3.99	73.46	9.46	83.61	10.35
o,p'-DDT	10-100	Y=1935.762X - 1711.88	0.999	3.29	9.98	91.81	6.73	97.61	3.20	95.32	4.77
Bifenthrin	10-100	Y= 8970.249X + 1680.584	0.999	1.35	4.08	87.65	2.51	93.41	0.86	92.62	2.16
Fenpropathrin	10-100	Y=437.6912X - 389.9147	0.999	1.27	3.86	78.14	6.76	93.07	1.68	93.37	3.90
Lambda-Cyhalothrin	10-100	Y=1697.897X - 956.2037	0.997	1.31	3.96	86.05	6.89	94.24	0.80	89.66	2.74
Permethrin-1	10-100	Y=1324.74X + 2606.145	0.998	2.94	8.90	96.67	13.17	96.81	2.14	94.30	2.43
Permethrin-2	10-100	Y=466.2425X + 2298.04	0.996	3.00	9.01	79.25	11.99	99.48	3.22	95.59	4.35
Cyfluthrin	10-100	Y=698.164X - 1985.532	0.998	1.77	5.36	72.28	5.17	90.53	4.07	87.78	4.69
Etofenprox	10-100	Y=10271.55X + 3184.196	0.999	1.53	4.64	88.85	6.51	96.87	2.54	91.50	2.07
Fenvalerate	10-100	Y=198.2861X - 995.5083	0.995	3.11	9.34	78.28	17.42	88.17	4.75	86.18	4.73
Deltamethrin	10-100	Y=616.1065X - 649.376	0.995	1.63	4.94	92.33	4.01	89.49	2.81	84.72	2.77
Acephate	10-100	Y=12157.7X-8002.79	0.998	2.33	7.78	85.84	18.91	70.37	8.13	89.98	6.34
Omethoate	10-100	Y=7405.37X-6211.61	0.998	0.77	2.32	76.37	18.54	99.02	12.77	72.85	11.39
Pymetrozine	10-100	Y=44682.3X-23144.5	0.999	1.16	3.85	80.94	20.01	91.39	7.87	80.38	7.39
Imidacloprid	10-100	Y=18244.4X+141512	0.999	1.10	3.66	105.37	15.02	84.54	8.66	95.40	8.63
Dimethoate	10-100	Y=64920.8X-34850.5	0.999	2.15	7.16	92.98	11.58	89.99	6.11	97.08	4.58
Carbendazim	10-100	Y=23666.1X+24909.9	0.998	0.31	1.05	97.09	20.10	83.15	7.68	77.08	8.22
Thiacloprid	10-100	Y=103350X-38492.3	0.999	0.31	1.04	92.26	9.62	100.81	4.23	83.77	3.16
Phosphomidon	10-100	Y=12686.8X-15515.8	0.999	0.95	3.16	90.35	12.00	92.52	6.24	91.21	4.82
Bendiocarb	10-100	Y=38449.1X-1468.49	0.999	0.70	2.11	91.44	10.44	87.29	6.44	74.46	5.16
Metribuzin	10-100	Y=8593.94X6464.76	0.998	0.67	2.22	112.42	15.05	98.32	7.72	72.12	7.25

Table 2 (continued)

Pesticide	Linear range (μgkg^{-1})	Regression equation	R^2	LOD (μgkg^{-1})	LOQ (μgkg^{-1})	Recovery (%) and RSD					
						0.01 mg kg^{-1}		0.05 mg kg^{-1}		0.10 mg kg^{-1}	
						Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Carbofuron	10-100	Y=80823.8X+46567.2	0.999	0.51	1.71	92.48	9.41	90.09	3.92	83.34	3.64
Carbaryl	10-100	Y=35821.8X-31173.9	0.999	0.52	1.74	117.17	10.01	90.28	6.38	80.18	7.73
Isoproturon	10-100	Y=7711.52X+576.106	0.999	0.39	1.29	93.73	14.38	88.79	5.61	83.42	7.21
Metalaxyl	10-100	Y=87543.8X-41343.9	0.999	1.89	6.29	96.44	7.14	83.59	4.44	79.51	5.27
Chlorantraniliprole	10-100	Y=4715.29X-3034.04	0.998	0.59	1.96	100.48	19.98	92.01	8.35	77.72	9.76
Coumatetryl	10-100	Y=82215.1X-33288.3	0.998	2.37	7.91	71.75	8.75	72.52	8.55	73.72	9.77
Paclobutrazole	10-100	Y=51008.9X+58212.6	0.999	2.70	8.99	93.08	12.91	90.64	6.68	85.96	7.01
Methoxyfenozide	10-100	Y=76275.8X-37752.8	0.999	0.38	1.25	101.25	8.39	87.88	4.92	95.69	4.69
Triademefon	10-100	Y=19644.8X+5928.25	0.998	2.53	8.44	95.20	10.34	97.25	4.82	88.77	6.28
Triazophos	10-100	Y=77553.0X -3885.1	0.999	0.47	1.56	97.84	10.23	89.13	5.73	80.86	5.63
Triademenol	10-100	Y=11495.1X-18874.0	0.999	0.71	2.37	77.88	20.01	90.74	8.19	97.48	7.32
Tetraconazole	10-100	Y=14025.1X-9830.14	0.999	2.16	7.18	87.05	11.65	119.42	5.09	84.39	5.49
Metalachlor	10-100	Y= 142100X-131314	0.999	0.60	2.01	93.52	6.34	87.75	4.04	91.64	4.05
Quinalphos	10-100	Y=23941.3X-9852.09	0.999	0.92	3.07	94.86	9.34	94.63	5.21	80.98	4.26
Penconazole	10-100	Y=39992.2+9137.19	0.999	1.08	3.58	90.07	11.32	94.21	4.73	79.25	3.51
Benalaxyl	10-100	Y=69608.2X-51006.3	0.999	0.53	1.78	97.00	7.77	87.62	4.68	79.44	5.83
Hexaconazole	10-100	Y=20745.7X-6112.43	0.998	1.76	5.86	96.95	13.44	96.37	5.16	75.41	4.86
Bitertenol	10-100	Y=4404.15X-7365.14	0.998	1.87	6.25	76.62	6.66	96.56	9.40	73.73	7.92
Phosalone	10-100	Y=9455.19X-21185.6	0.999	0.54	1.79	76.41	19.06	97.40	6.58	96.13	6.26
Spinosad	10-100	Y=16691.6X-723.809	0.999	1.16	3.87	75.36	14.32	72.14	7.86	79.67	8.05
Thiobencarb	10-100	Y=3352.48X+11349.4	0.998	0.93	3.11	108.44	4.53	89.43	14.25	86.65	9.74
Difenconazole	10-100	Y=37189.4X-10403.9	0.998	1.29	4.29	93.38	9.55	91.50	4.76	76.21	5.12
Pretilachlor	10-100	Y=189133X-52680.2	0.999	0.71	2.36	99.07	9.15	89.51	5.57	79.98	3.74
Profenofos	10-100	Y=7001.79X+7217.76	0.998	0.88	2.65	115.35	8.67	118.13	6.25	89.25	8.39
Emamectin benzoate	10-100	Y=21577.7X-18433.8	0.999	1.79	5.98	98.97	12.53	92.94	4.09	71.40	6.17
Buprofezin	10-100	Y=114731X+219535	0.998	2.97	9.89	99.4	7.70	87.20	4.67	93.13	4.67
Hexythiazox	10-100	Y=12364.0X+24909.9	0.998	1.09	3.29	84.4	14.49	94.56	6.80	94.31	5.21
Pendimethalin	10-100	Y=10796.8X-25506.8	0.998	1.44	4.79	74.99	17.20	88.68	9.02	78.49	10.23
Fenpyroximate	10-100	Y=79117.6X+24861.3	0.998	1.65	5.52	103.25	7.81	82.41	4.39	84.56	4.13

(Table 2). The recovery of 71.07 to 115.35% was for fenitrothion and allethrin at 0.01 mgkg^{-1}; 70.00 to 115.95% for allethrin and p,p'-DDD at 0.05 mgkg^{-1} and 83.61 to 120.50% for endosulfan sulfate and allethrin for 0.1 mgkg^{-1} level spike and recovery for pesticides analyzed with GC techniques. For all levels of recovery, the percent RSD was found acceptable range (≤ 20.00%), and measurement of uncertainty was found within an acceptable range of 9.03 to 15.23 μgkg^{-1} at 50 μgkg^{-1} spiking level for pesticides tested with GC techniques (Table 3).

Furthermore, the pesticide tested with LC techniques recorded their recovery in the range of 71.75 (coumatetryl) to 117.17% (carbaryl) at 0.01 mgkg^{-1} (LOQ level); 70.37 (acephate) to 119.42% (tetraconazole) at 0.05 mgkg^{-1} (5 times of LOQ); 71.40 (emamectin benzoate) to 97.48% (triademenol) at 0.01 mgkg^{-1} (10 times of LOQ) spike and recovery level with relative standard deviation (RSD) was less than 20%. The calculated measurement of uncertainty was in the range of 7.00 to 15.83 μgkg^{-1} at 50 μgkg^{-1} and found within the acceptable limit. The recovery obtained in the present study complied with the method validation guidelines SANTE/12682/2019 guidelines.

Intraday precision test for the method was determined by spiking the middle concentration (0.05 mgkg^{-1}) from the matrix match linearity was found acceptable with the obtained recovery of 70.37 to 119.42% and RSD of 4.05 to 13.34% for

Table 3 Repeatability, ruggedness, matrix effect, measurment uncertainty

Pesticide	Repeatability (0.05 mg kg^{-1})	% RSD (n=6)	Reproducility 0.05 mg kg^{-1} (n=6)% RSD comparison with changed date and analyst	Matrix effect (%)	Uncertainty of Measurement (±) at 50 μg kg^{-1}			MRL (mg kg^{-1})	
					Combined Uncertainty (Uc)	Coverage factor (k)	Expanded Uncertainty ($U_{exp.}$)	EU	FSSAI
Phenol, 4-bromo-2-chloromo	77.44	3.20	7.21	-7.08	5.48	2	10.96	-	-
Trifluralin	88.66	4.77	2.03	-6.06	6.16	2	13.23	0.01	-
Alpha-BHC	84.54	3.82	1.67	-4.75	6.41	2	12.82	0.01	-
Beta-BHC	86.46	4.62	2.14	-4.75	5.83	2	11.67	0.02	-
Diazinon	85.45	6.70	0.36	-2.98	6.21	2	12.42	-	-
Fluchloralin	89.80	9.29	2.77	-4.56	5.76	2	11.53	0.10	-
Tri-allate	81.07	5.80	5.22	-3.45	6.77	2	13.54	-	0.2
Iprobenfos	86.17	2.10	0.48	5.26	5.38	2	10.77	-	0.05
Chlorpyrifos-methyl	83.42	4.02	1.94	-4.37	6.38	2	12.75	0.20	0.01
Parathion-methyl	89.96	5.48	2.80	7.59	5.94	2	11.88	-	-
Alachlor	87.47	2.76	0.11	-3.88	5.49	2	10.99	0.01	-
Heptachlor	84.66	5.17	1.62	7.23	7.09	2	14.18	0.50	-
Fenitrothion	84.11	6.54	0.70	3.40	5.43	2	10.86	-	-
Aldrin	78.10	7.51	7.86	-3.68	7.21	2	14.43	0.05	0.5
Chlorpyrifos	88.49	5.96	2.16	-1.43	7.21	2	14.43	0.05	0.01
Parathion	90.19	6.83	2.65	8.77	5.69	2	11.39	0.05	-
Chlorfenvinphos (E)	84.00	3.68	2.09	7.95	5.18	2	10.35	-	-
Phenthoate	84.14	3.57	2.74	7.42	4.51	2	9.030	-	0.05
Butachlor	97.64	3.01	2.57	4.54	4.63	2	9.260	-	-
p,p'-DDE	87.12	3.18	4.34	0.40	4.69	2	9.380	0.01	-
Endrin	76.76	7.18	1.62	6.95	6.09	2	12.18	0.50	-
Beta-Endosulfan	70.98	12.41	5.07	-0.79	5.63	2	11.26	0.05	-
p,p'-DDT	87.05	2.18	3.91	7.58	6.39	2	12.78	0.50	-
Endosulfan sulfate	74.15	9.87	8.11	1.02	5.55	2	11.10	0.05	-
o,p'-DDT	88.85	4.08	8.22	20.92	7.62	2	15.23	0.05	0.05
Bifenthrin	89.52	2.50	3.57	1.16	6.21	2	12.43	-	0.03
Fenpropathrin	86.07	3.77	5.08	1.39	4.60	2	9.210	0.20	1.00
Lambda-Cyhalothrin	89.19	3.89	5.32	7.18	5.55	2	11.11	0.05	-
Permethrin-1	89.00	4.57	6.80	-8.08	6.67	2	13.34	0.02	-
Cyfluthrin	85.67	9.91	4.73	-8.35	6.46	2	12.92	-	0.01
Etofenprox	89.08	2.48	6.31	-9.67	6.58	2	13.16	-	-
Fenvalerate	85.01	9.83	1.30	-11.04	6.14	2	12.28	0.05	2.00
Deltamethrin	89.06	3.09	1.84	-15.39	6.98	2	13.97	0.05	2.00
Acephate	70.37	10.48	10.57	-18.88	7.50	2	15.00	0.02	1.00
Benalaxyl	87.62	4.77	14.07	-11.51	6.89	2	13.78	0.05	-
Bendiocarb	87.29	6.39	4.48	-11.99	4.44	2	8.88	-	-
Bitertenol	96.56	9.84	8.25	-21.75	4.50	2	9.00	0.05	-
Buprofezin	87.20	4.71	5.33	-9.68	3.59	2	7.18	-	0.05
Carbaryl	90.28	6.36	9.08	-20.70	4.48	2	8.97	1.00	2.50
Carbendazim	83.15	10.45	6.07	-10.12	4.17	2	8.34	0.10	0.1
Carbofuron	90.09	3.88	8.04	-19.89	5.10	2	10.20	0.10	-
Chlorantraniliprole	92.01	8.32	6.05	-17.80	7.51	2	15.02	-	0.40
Coumatetryl	73.04	8.03	7.98	-5.04	3.93	2	7.87	-	-

Table 3 (continued)

Pesticide	Repeatability (0.05 mg kg^{-1})	% RSD (n=6)	Reproducility 0.05 mg kg^{-1} (n=6)% RSD comparison with changed date and analyst	Matrix effect (%)	Uncertainty of Measurement (±) at 50 μg kg^{-1}			MRL (mg kg^{-1})	
					Combined Uncertainty (Uc)	Coverage factor (k)	Expanded Uncertainty ($U_{exp.}$)	EU	FSSAI
Difenconazole	91.50	4.88	6.34	7.52	3.93	2	7.87	-	-
Dimethoate	89.99	6.30	5.46	-14.10	4.15	2	8.30	0.02	-
Emamectin benzoate	92.94	4.48	8.06	17.91	3.81	2	7.62	-	-
Fenpyroximate	82.41	4.57	3.89	18.03	5.11	2	10.23	-	-
Hexaconazole	96.37	5.70	5.14	-19.47	3.54	2	7.10	0.10	0.02
Hexythiazox	94.56	6.99	8.24	-22.05	3.70	2	7.41	-	-
Imidacloprid	84.54	9.03	4.77	3.31	4.88	2	9.75	-	0.05
Isoproturon	88.79	5.83	11.60	-11.20	4.25	2	8.51	0.05	-
Metalachlor	87.75	4.05	7.37	-29.73	4.40	2	8.80	-	-
Metalaxyl	83.59	4.65	4.74	-15.91	4.95	2	9.91	0.05	-
Methoxyfenozide	87.88	4.77	6.84	1.25	4.48	2	8.96	-	-
Metribuzin	98.32	7.77	10.21	-6.97	3.50	2	7.00	-	-
Omethoate	99.02	12.87	13.76	-9.48	3.87	2	7.75	0.20	-
Paclobutrazole	90.64	6.21	15.29	-3.05	4.37	2	8.74	-	-
Penconazole	94.21	5.60	4.09	-22.19	3.71	2	7.43	0.05	-
Pendimethalin	88.68	8.72	6.16	-11.54	4.33	2	8.66	-	0.05
Phosalone	98.12	7.10	7.38	9.35	3.55	2	7.11	1.00	-
Phosphomidon	92.52	5.97	5.54	8.27	3.92	2	7.85	0.15	-
Pretilachlor	89.51	5.45	10.98	-13.50	4.25	2	8.51	-	0.05
Profenofos	118.13	5.27	10.10	15.18	7.48	2	14.96	0.05	-
Pymetrozine	91.39	9.27	3.48	-19.79	4.04	2	8.09	0.50	0.01
Quinalphos	94.63	5.36	3.58	-18.77	3.69	2	7.38	0.05	0.01
Spinosad	75.81	4.92	4.79	-16.03	6.380	2	12.76	-	-
Tetraconazole	119.42	4.33	5.50	-11.13	7.92	2	15.83	-	-
Thiacloprid	100.81	4.53	10.49	-28.10	3.55	2	7.10	-	0.02
Thiobencarb	89.43	13.34	13.75	-15.91	4.64	2	9.28	-	-
Triademefon	105.95	4.54	5.97	-18.98	3.54	2	7.09	0.30	-
Triademenol	90.74	6.89	4.68	-11.17	4.08	2	8.15	0.30	-
Triazophos	89.13	5.79	8.94	22.39	4.28	2	8.57	0.02	0.60

RSD Relative standard deviation; *EU* European Union; *FSSAI* Food Safety Standard Authority of India

pesticides tested with LC-MS/MS and 76.76 to 97.64% with RSD of 2.10 to 19.87% for pesticides tested with GC-MS/MS and complained within the acceptable range for relative standard deviation < 20%. Interday precision carried out in blank rice matrix spiked at 0.05 $mgkg^{-1}$ (5 times of LOQ) and analyzed on different days and change of analyst revealed less than 20% RSD for pesticides tested with LC-MS/MS and GC-MS/MS. With these tests, the optimized method was found suitable and had reproducibility with the rice matrix and can be employed for analysis of 72 different pesticides in rice and other cereal matrices.

With respect to the matrix effect, the highest matrix-induced signal enhancement effect was obasevred for metalochlor (22.39%), and triazophos showed the lowest matrix-induced signal suppression effect (−29.73%). To know the exact matrix effect on pesticide detection and quantification, the matrix linearity curve was compared with the pure solvent standard curve. Table 2 depicts that the rice matrix has a strong influence on the analytical signals of the tested pesticides in the study. It has been noted that both positive and negative matrix effect in rice. The strength of this matrix effect depends on the different properties of the pesticide viz.,

physiochemical, molecular mass, thermal stability, and temperature at which boiling analytes (Aparecida de Sousa et al. 2012). The negative matrix effect is observed in most pesticides due to the degradation of pesticides during analysis either during extraction. When the sample is injected, there is a competition between pesticide and other matrix components, such that enhancement of the signal was also observed. Thus the comparison between the pesticides in the sample matrix and the standard solution is characterized by a positive matrix effect for some of the pesticides studied viz., parathion, chlorfenvinphos, phenthoate, butachlor, pp-DDE, endrin, pp-DDT, o, p- DDT, bifenthrin, fenpropathrin, lambda-cyhalothrin. (Tsipi et al. 2015; Stahnke and Alder 2015)

Similar method reported previously for analysis of 200 pesticides in cereals using GC-MS/MS found acceptable recovery but did not record on the uncertainty measurement (He et al. 2015). A method was developed in the rice matrix using HPLC-PDA showed recovery in the range of 74–127% for 9 different pesticides and real samples analysis showed residue below quantification limit of 0.6 mg/kg (Tsochatzis et al. 2010). Present work is in agreement with the previous reports on the analysis of multiclass pesticides in wheat, barn and corn using GC-MS/MS and provided the compliance to the requirements for EU MRLs (Walorczyk 2007; Hernandez et al. 2013). Furthermore, the developed method is in line with methods reported previously for analysis of more than 90 pesticides in rice matrix had similar observation to the present findings (Anastassiades et al. 2003; Libin et al. 2006; Liu et al. 2007; Takatori et al. 2008; Zhang et al. 2011; Mastovska et al. 2009; Nguyen et al. 2008; Pengyan et al. 2006). The analysis of multi-residues in brown rice, orange, and spinach (Lee et al. 2018); rice and wheat flour (Grande-Martínez et al. 2016) and sulfonylurea herbicides in cereals (Ni et al. 2018) are some of the recent methods having similar results but lack of a statistical procedure on measurement of uncertainty. The present method addressed this research gap and explain about the procedure for estimation of measurement uncertainty which is very much required for decision making and report of results in food contaminant analysis.

Calculation of Measurement Uncertainty

The expanded uncertainty (U_{exp}) at a 95% confidence level was calculated by the addition of a coverage factor of 2 for the fortification level of 0.05 μg g^{-1}. The combined uncertainty was in the range of 3.50 to 7.97 μg, and expanded uncertainty was in the range of 7.00 to 15.83 μg. The values reported in expanded and combined uncertainty were below the acceptable range of ± 30.00 μg g^{-1} at a fortification level of 0.05 μg g^{-1}. Different sources of uncertainty due to repeatability, balance for weighing of sample and standards, volumetric flask for preparation of 1000, 100 and 10 μg mL^{-1} stock, intermediate and working solution, micropipette for preparation of calibration curve, recovery of particular analyte and certified reference material were contributed to the combined uncertainty (Uc) and expanded uncertainty (U_{exp}) and found accurate in recording the values set in them for sample preparation. Therefore, it is concluded that the optimized method efficient enough and suitable for the determination of pesticide in different cereal matrices using LC-MS/MS and GC-MS/MS (Table 2).

Method Suitability for Other Cereal Matrices

The validated method was employed to know suitability with other cereal matrices such as paddy, polished rice, rice bran, and wheat flour. These matrices havrecorded the recovery in the range of 78.11 to 136% for the paddy, 70.18 to 143.34% for polished rice, 73.60 to 157.80% for rice bran, and 73.76 to 120.94% for wheat flour. In paddy samples, the recovery of few pesticides *viz., chlorpyrifos*-methyl (120.15%), chlorpyrifos (124.18%), pp-DDE (121.09%), carbendazim (136.28%), phosolone (123.53%) found above 120%. Among 72 pesticide compounds, 96.25% were found acceptable recovery with the method followed for extraction. Whereas in polished rice, carbaryl (120.77%), phosolone (121.48%), triazophos (122.78%) were found out of acceptable recovery range (70–120%), and more than 95% of the pesticides were found acceptable. The extraction method was suitable for 62 pesticides in rice bran samples and 10 pesticides reported as outlier recovery, which contributed to13.88%. Whereas only two pesticides found the residues above 120% in the wheat matrix and 97.22% compound tested was found acceptable recovery (Table 4). The present method fulfills the requirements of the validation guidelines SANTE/12682/2019, which stated that the method validated in any of the representative matrices of the group of commodities would hold good for other similar matrices.

Proficiency Testing in Cereal Products

Acephate, buprofezin, chlorantraniliprole, deltamethrin, and quinolphos were quantified in the PT samples (rice) and reported. The *Z* score for the pesticide reported in rice powder was in the range of −0.68 to 1.81 and found satisfactory. Similar results were recorded in wheat flour for five different pesticides such as imidacloprid (0.96), bifenthrin (−0.21), chlorpyrifos (1.03), quinolphos (0.94), and buprofezin (−0.21). The proficiency test indicated that the developed and validated method is highly sensitive, accurate, and can be used to analyze pesticide residues in grains and related matrices (Table 5).

Table 4 Performance of the proposed method in terms of recovery in different cereal matrices

Pesticide	Recovery (%) in different cereal matrices at 0.05 mg kg^{-1}			
	Paddy	Polished rice	Rice bran	Wheat
Phenol, 4-bromo-2-chloromo	79.00	78.00	78.00	73.76
Trifluralin	87.26	94.49	102.03	89.81
alpha-BHC	103.34	75.42	73.60	83.38
beta-BHC	118.23	111.92	77.16	83.31
Diazinon	114.36	92.53	100.76	93.31
Fluchloralin	110.60	98.45	102.47	91.46
Tri-allate	118.72	91.58	101.66	88.00
Iprobenfos	117.37	97.92	102.58	98.72
Chlorpyrifos-methyl	120.15	93.34	101.59	91.82
Parathion-methyl	111.17	95.13	100.41	92.16
Alachlor	109.28	91.00	95.78	95.53
Heptachlor	78.11	103.64	82.53	75.86
Fenitrothion	103.62	70.18	75.98	77.19
Aldrin	114.80	88.40	93.31	85.99
Chlorpyrifos	124.18	95.89	101.86	93.19
Parathion	112.37	95.85	104.46	97.01
Chlorfenvinphos	101.76	101.30	104.95	93.99
Phenthoate	94.50	86.29	89.90	88.47
Butachlor	111.55	82.75	88.63	78.20
p,p'-DDE	121.09	96.35	93.93	85.15
Endrin	113.49	104.65	103.65	87.55
beta-Endosulfan	93.26	107.56	100.44	85.14
o,p'-DDT	108.79	90.87	83.83	85.07
Endosulfan sulfate	108.87	109.01	97.17	81.31
p,p'-DDT	107.01	117.01	114.39	45.76
Bifenthrin	110.21	100.26	91.10	92.58
Fenpropathrin	103.63	101.48	97.88	96.54
lambda-Cyhalothrin	98.88	103.28	94.64	90.24
Permethrin	103.39	94.57	91.96	91.19
Cyfluthrin	105.96	100.19	101.59	93.12
Etofenprox	99.44	101.67	97.88	92.05
Fenvalerate	94.16	102.31	101.13	85.80
Deltamethrin	87.26	101.69	98.23	88.97
Acephate	97.26	95.29	117.37	74.07
Benalaxyl	92.67	101.24	106.91	120.94
Bendiocarb	108.97	108.29	105.32	107.87
Bitertenol	84.86	101.82	113.95	95.99
Buprofezin	116.38	113.81	116.51	109.30
Carbaryl	109.44	120.77	112.16	99.94
Carbendazim	136.28	113.34	142.68	96.32
Carbofuron	110.82	115.81	118.58	106.18
Chlorantraniliprole	106.76	101.61	125.59	103.79
Coumatetryl	118.02	116.60	113.69	116.36
Difenconazole	76.98	91.08	108.05	112.02
Dimethoate	106.37	112.44	113.48	115.89
Emamectin Benzoate	93.07	105.92	96.66	118.46
Fenpyroximate	103.91	113.05	119.74	104.66

Table 4 (continued)

Pesticide	Recovery (%) in different cereal matrices at 0.05 mg kg^{-1}			
	Paddy	Polished rice	Rice bran	Wheat
Hexaconazole	96.77	95.54	121.70	108.02
Hexythiazox	112.95	110.91	114.67	100.33
Imidacloprid	88.65	92.55	95.90	94.73
Isoproturon	78.20	98.36	105.78	109.03
Metalachlor	102.02	106.88	114.01	115.24
Metalaxyl	106.52	116.97	113.04	112.30
Methoxyfenozide	108.16	117.59	137.03	110.72
Metribuzin	114.95	117.38	133.92	109.24
Omethoate	78.61	84.56	87.67	86.75
Paclobutrazole	95.76	99.88	109.99	120.04
Penconazole	97.54	98.42	100.72	103.71
Pendimethalin	98.54	100.79	157.80	101.38
Phosolone	123.53	121.48	115.27	110.69
Phosphomidon	103.97	114.56	104.03	114.03
Pretilachlor	103.92	108.33	116.38	109.78
Profenofos	107.66	113.88	143.21	99.36
Pymetrozine	79.11	77.12	85.06	78.77
Quinalphos	98.19	104.69	102.47	106.61
Spinosad	102.40	101.63	122.07	107.56
Tetraconazole	100.24	92.15	115.70	92.85
Thiacloprid	95.80	105.15	114.93	103.35
Thiobencarb	116.34	119.86	101.17	107.88
Triademefon	99.77	100.36	87.89	119.07
Triademenol	82.10	98.01	125.49	120.53
Triazophos	113.22	122.78	131.88	108.78

Method Application to Real Samples

Among 100 filed collected paddy samples, 24% were found contaminated with buprofezin and profenofos, 11% with imidacloprid, 5% with chlorantraniliprole, 8% with bifenthrin, 23% with acephate, 8% with cypermethrin, and 13% with carbendazim. The three pesticides were found above their prescribed MRL and remaining found below the MRLs. The

Table 5 Results of proficiency testing ('*Z*'-scores) for the pesticides in rice and wheat powder samples analyzed with proposed method

Name of the pesticides	Assigned values ($mgkg^{-1}$)	Reported value ($mgkg^{-1}$)	'*Z*' Score	Performance
Rice				
Acephate	1.342	1.422	0.19	Satisfactory
Buprofezin	0.496	0.706	1.52	Satisfactory
Chlorantraniliprole	0.425	0.557	0.99	Satisfactory
Deltamethrin	0.358	0.291	-0.68	Satisfactory
Quinolphos	0.880	1.325	1.81	Satisfactory
Wheat powder				
Imidacloprid	0.1470	0.185	0.96	Satisfactory
Bifenthrin	0.1888	0.178	-0.21	Satisfactory
Chlorpyrifos	0.4454	0.575	1.03	Satisfactory
Quinolphos	1.0785	1.364	0.94	Satisfactory
Buprofezin	0.0738	0.070	-0.21	Satisfactory

Table 6 Pesticide residues detected monitoring samples of paddy (n=100) and rice (n=50) collected from farmers field and processing mills

Pesticides detected in paddy	Contaminated samples (%)	Max. quantified (mg kg^{-1})	MRL (mg kg^{-1}) (As per EU)	Pesticides Detected in rice	Samples contaminated (%)	Max. quantified (mg kg^{-1})	MRL (mg kg^{-1}) (As per EU)
Buprofezin	24.0	0.338	1.00	Carbendazim	2.0	0.140	1.00
Profenophos	24.0	0.017	0.05	Permethrin	4.0	0.034	0.05
Imidacloprid	11.0	0.037	0.50	Imidacloprid	0.5	0.090	0.50
Chlorantraniliprole	5.0	0.128	NA	Chlorantraniliprole	0.5	0.031	NA
Bifenthrin	8.0	0.147	0.05	Buprofezin	1.5	0.030	1.00
Acephate	23.0	0.315	0.02	Deltamethrin	1.5	0.012	0.05
Cypermethrin	8.0	0.162	0.05				
Difenconazole	1.0	0.003	NA				
Carbandazim	13.0	0.447	0.1				

paddy is being subjected to various processing before being consumed as rice, and the residues observed above the MRLs in the unhusked grains may be removed during processing. The developed method is well suited for screening the pesticides during the harvest of the paddy grains and intern inform about the pesticide usage pattern and contamination by various other means.

The analysis of rice samples collected from different industries revealed the presence of carbendazim, permethrin, imidacloprid, chlorantraniliprole, buprofezin, deltamethrin in 2.0, 4.0, 0.5, 0.5, 1.5, and 1.5% of samples, respectively, while they have recorded mean maximum concentration of 0.064, 0.160, 0.090, 0.031, 0.021, and 0.011 $mgkg^{-1}$ of residues, respectively. When compared with the FSSAI and the EU MRLs, none of the chemicals found more than MRLs. The developed method is highly sensitive and suitable for the simultaneous determination of multi-class pesticides in processed rice samples (Table 6). A similar approach has been made in previous work on the application of the developed method for detection of pesticides such as dichlorvos and isoprothiolane in rice samples (He et al. 2015) and multi-class pesticide residue analysis revealed the presence alpha-endosulfan (0.1 μgg^{-1}), deltamethrin (0.067 μgg^{-1}), and fipronil (0.01 μgg^{-1}) (Uddin et al. 2011; Tomer 2013); chlorpyrifos in rice samples (Zhang et al. 2012) proved that the efficiency of the developed method.

Comparison of the proposed method superiorities over other works includes the following: (i) over 72 pesticides were determined rice and other by-products and wheat matrices using GC-MSMS and LC-MSMS, whereas in other paper except few studies involving less sensitive techniques (ii) Estimated the uncertainty of measurement for target analytes at 50 mg/kg spiking level which every analytical method failed to report this statistical procedure which helps in decision making and reporting results (iii) method validation using two superior techniques such as LC-MS/MS and GC-MS/MS was achieved and (iv) residues detected in field samples has proved reproducibility of the method.

Conclusion

The multi-residue analytical method for simultaneous determination of 72 different pesticide residues in rice matrix is highly sensitive, and can detect the pesticides at five times lower (0.002 $mgkg^{-1}$) the values of tolerance limits (MRLs) given by the European Union and Food Safety Standard Authority of India. This method is suitable for screening 72 different chemical pesticides in other cereal matrices in short run time. Combined Uncertainty (Uc) and Expanded Uncertainty (U_{exp}) were found accurate and within the limit. The method was verified through checking the proficiency test samples, and the Z score was found within the acceptable range of −2 to +2. This method has got high accuracy (within 70–120%), precision, reproducibility, and ruggedness suitable for monitoring pesticide residues in grain cereals and its products.

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Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Informed consent does not apply to this study.

Conflict of Interest Harischandra Naik R declares that there is no conflict of interest. Pallavi M S declares that there is no conflict of interest. Pavan Kumar K declares that there is no conflict of interest. Vanitha B.K declares that there is no conflict of interest. Chandra Sekhara Reddy V declares that there is no conflict of interest. Shwetha A declares that there is no conflict of interest. Nandini Nandini declares that there is no conflict of interest. Naveen Kumar declares that there is no conflict of interest. Udaykumar Nidoni R declares that there is no conflict of interest. Bheemanna M declares that there is no conflict of interest.

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