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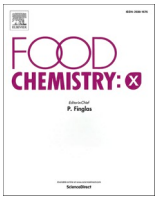

Determination of 73 multi-class pesticides in okra (*Abelmoschus esculentus* L.) fruits using LC–MS/MS and GC–MS/MS and estimation of analytical uncertainty of measurement

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ABSTRACT

This study developed a method to simultaneously determine 73 multi-class pesticides in okra fruit using LC-MS/MS and GC–MS/MS. The sample was extracted with acetonitrile and subsequent clean-up through dispersive-SPE method. The quantification level of the technique was 0.01 μg g^{-1} and compliance to the MRLs fixed by the regulatory bodies like EU and FSSAI. The recovery at 10, 50, and 100 μg kg^{-1} spiked levels; intra and inter-day precision at 50 μg kg^{-1} were found within 70–120% with RSD less than 15% with LC-MS/MS and GC–MS/MS. Measurement uncertainty was in the range of 1.81 to 12.91 μg kg^{-1} estimated at 50 μg kg^{-1}. The matrix effects were slightly higher for LC than GC-compatible pesticides. Risk assessment for pesticides detected in the field and market samples found no hazardous to the consumers except profenofos. The proposed method is highly sensitive, reproducible for the complex matrix like okra, and meets the regulatory standards.

1. Introduction

Human diets are incomplete without vegetables. They constitute a significant part of the preparation of various foods. Vegetables are a good source of essential nutrients required for balanced diets *viz.,* vitamins (A, B1, B6, B9, C, and E), carbohydrates, proteins, antioxidants (sulphoraphane, nasunin, allicin, and diosgenin), and minerals (Klein, 1987; Naik Rathod et al., 2021). A vegetable diet reduces the risk of several diseases and treats different diseases (Slavin & Lloyd, 2012). India is the leading vegetable producing country with an area of 10,259 (000 ha) and production of 1, 84,394 (000 tones), and productivity of 17.70 tones/ha (Indian Horticulture Database, 2018). Vegetable crops use about 12–13% of total pesticides in agriculture, and most of them are synthetic pesticides. Pesticide usage is a common practice in vegetable production due to visible knockdown effects on insect pests resulting in toxic residues in final produce (Kumari & John, 2005). The pesticide residues in agricultural produce, water, and environment samples leading to pesticide poisoning (Darko & Akoto, 2008). Lack of awareness of toxic effects, indiscriminate use leads to pesticide residues in vegetables. Chemical residues above the tolerance level (MRL) may contribute the potential health hazards on chronic exposure and threat to food safety (Tang et al., 2018; Radwan et al., 2015; Hingmire, Oulkar, Utture, Shabeer, & Banerjee, 2015). Thus, pesticide residues quantification in various foods, either raw or processed, is a critical international trade requirement for consumers (Hingmire et al., 2015).

Among the vegetables, okra, *Abelmoschus esculentus* (L.) is a high export potential vegetable grown in India. India is a leading producer of okra fruits with a total production of 6.47 million tons (3.9%) from 5.28 million hectares of the cultivated area (5.7%) with productivity of 11.63 metric tons per hectare (Indian Horticulture Database, 2018). It is valued for its green fruits, rich in proteins, calcium, phosphorus, iron, carotene, and vitamins. About 72 insect pests are recorded in the okra crop from germination to harvest. Among pests, leafhopper, whitefly, shoot, and fruit borers were collectively causing 36–90% of yield loss

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(Pal et al., 2016; Gupta et al., 2009). To avoid losses, synthetic chemical pesticides are the first choice for farmers due to their high biological efficacy and immediate effect. Farmer's primarily relies on new generation chemicals like neonicotinoids (Gurbuz et al., 2014), diamide insecticide (Paramasivam & Bhuvaneswari, 2021), and pyrethroid insecticides are very common and frequently applied since planting vegetables and are of great concern for environmental safety (Nafees & Jan, 2009; Bhandari, Atreya, Yang, Fan, & Geissen, 2018). Tender fruits of okra are harvested every 2–3 days with non-compliance to pre-harvest intervals, and due to its short fruits plucking periods, pesticide residue may likely occur (Paramasivam & Bhuvaneswari, 2021). A great necessity of screening the vegetable foods for pesticides before being subjected to export demands high throughput techniques to develop the analytical methods.

Pesticide residues in the harvested vegetable produce beyond their tolerance limits (MRL) are the trade barriers at the export point and non-compliance to the food safety regulations (FSSAI, 2018; European Commission, 2008). MRLs are very low for most of the pesticides required an efficient method to meet global food standards. A variety of analytical methods were reported for the simultaneous quantitative analysis of pesticides in fruits and vegetables *viz.,* GC with ion trap (Savant et al., 2010); GC-ToF MS and LC-MS/MS (Banerjee et al., 2008 and 2012; Walorczyk, 2008), LC-APCI-MS/MS for multi-class pesticides in rice (Caldas et al., 2011); LC-MS/MS in spices (Yogendrarajah, Van, De Meulenaer, & De Saeger, 2013); LC-MS/MS & GC–MS/MS for multiresidue in pigeonpea (Naik et al., 2021) and rice (Harischandra et al., 2021) employed for rapid detection and quantification of residues. For an analytical method, it is very essential to have the uncertainties associated with it that greatly influence the analytical results for making a decision to the real samples. Most of the previous work has lack of measurement uncertainties. For a rapid detection of pesticide residues in vegetable foods is always challenging due to the complex matrix nature. This is achieved following suitable methods, and modifications provide solutions to practical applications to the complex matrices. In this study, quick, sensitive, and reproducible analytical methods using LC-MS/MS and GC–MS/MS were developed and validated in okra fruits following European commission validation guidelines. The technique was applied to real sample analysis, estimated the associated uncertainty, and assessed the risk of consuming vegetables detected with pesticides.

2. Material and methods

2.1. Pesticide selection

Seventy-three pesticides were selected in this study, which included both GC and LC suitable molecules. Certified reference materials (CRM) with known purity (≥98.0%) having traceability to ISO/IEC 17,034 were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The names of these pesticides are presented in Table S1.

2.2. Chemical, reagents and apparatus

The solvents (>99.9% purity) *viz.,* methanol, ethyl acetate, and acetonitrile (LC-MS grade) were procured from Merck (Mumbai, India). Anhydrous sodium chloride, magnesium sulfate, sodium acetate, and sodium sulfate (>99% purity) were purchased from Himedia (Bangalore, India). Ammonium formate and formic acid (90% purity) were obtained from Empart (Hyderabad, India). Primary secondary amine (PSA, 40 μm size) was purchased as dispersive-solid phase extraction sorbent from Agilent make (Agilent Technologies India Pvt. Ltd., Bangalore, India). Ultrapure water with a resistivity of 18.2 M_{Ω} was collected from a Milli-Q (Merck Millipore, USA) water purification system installed in the laboratory.

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.

2.3. Standard solutions

Stock solutions of each pesticide compound were weighed (10 ± 0.1 mg) using a calibrated analytical balance (7 digit microbalance: Sartorius®) in a volumetric (10 mL) flask and dissolved with 10 mL methanol for LC and ethyl acetate for GC compatible. Further, the final concentration in the primary stock solution was calculated using the formula:

$$\text{Concentration}\left(\mu g\ mL^{-1}\right) = \frac{\text{Weight of CRM (mg)} \times \text{Purity of CRM (\%)} \times 1000}{\text{Final volume (mL)} \times 100}$$

All the solutions were prepared in different volumes calibrated class 'A' glassware. An intermediate stock solution of 100 μg mL^{-1} was prepared, drawing 1 mL of standard stock solution (1000 μg mL^{-1}) into a 10 mL volumetric flask and volume made-up. Working standard mixture (10 μg mL^{-1}) was prepared by mixing a known amount of individual solution through serial dilution techniques. All these solutions were stored in a refrigerator at −20 °C. The calibration standard solution ranging from 0.01 to 1.0 μg mL^{-1} was prepared and injected to LC-MS/MS and GC–MS/MS. The matrix match standards having similar linear concentrations were prepared simultaneously using untreated okra fruit.

2.4. Sample preparation

Approximately 2 kg okra fruits (pesticide-free) were chopped, homogenized (Robo-Coup), and collected in a cleaned inert container. A test portion (10 g) of the sample was transferred into 50 mL centrifuge tubes, and acetonitrile (20 mL) was added and homogenized using a homogenizer for 3 min. Then 1.5g NaCl was added and vigorously shaken 30 *sec* and centrifuged the content at 13416xg for 10 min. The upper organic layer (10 mL) was carefully transferred into a test tube containing the 5g anhydrous Na_2SO_4 to remove the moisture. For d-SPE clean-up, 8 mL of extract was transferred into a centrifuge tube containing 200 mg PSA and 1.0 g anhydrous MgSO4 added. The tubes were shaken well and centrifuged 13416xg for 5 min to get a clear solution. The supernatant (1 mL) solution was filtered through a 0.2 μm syringe filter and directly injected (2 μL) into LC-MS/MS. In GC–MS/MS analysis, 2 mL of the clear supernatant was concentrated using a nitrogen flash evaporator, reconstituted with 1.0 mL ethyl acetate, and filtered into GC vials using a 0.22 μm PTFE nylon filter, and 1 μL was injected into GC–MS/MS.

2.5. LC-MS/MS parameters

LC-MS/MS (LC-MS 8040, Shimadzu®) system attached with UHPLC for separation and triple quadrupole detector was used to confirm and quantify multi-class pesticides in okra fruits. The instrument was controlled with LabSolution® software, version 1.5. Chromatographic separation of pesticides (39) done with C18 column (Octadecylsilyl; 2 mm i.d × 150 mm, 2.2 μm particle size). Binary gradient program with 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 (B) consisted of 5 mM ammonium formate, 0.01% formic acid made up to the volume of 100 mL with 100% methanol. Flow rate and injection volume of 0.4 mL min^{-1} and 2 μL, respectively was maintained throughout the study. The mobile phase gradients program for the binary pump was initially 60% A and 40% B for 15 min. followed by 100% B for five min. and then 60% A and 40% B for 5 min. The column temperature was maintained at 40 °C. The cycle time and total time program were 0.234 *sec* and 19 min.,

respectively. The mass spectrometer was operated in electrospray ionization (ESI) with both positive and negative modes. At the same time, maximum compounds were separated through ESI positive mode. The triple quadrupole mass was employed for analyte fragmentation with optimized voltage and collision energy to get the highest sensitivity. The specific MS/MS parameters such as interface current (0.1 μÅ), heat block temperature (400 °C), and desolvation line temperature (250 °C) were optimized and acquired the MS data. High pure nitrogen (99.99% purity) was used as nebulizer and drying gas at 2.9 and 15 L min^{-1}, respectively. High pure argon gas (99.99% purity) 230 kpa was used for collision energy diffraction (CID). Mass scan speed (6000 $usec^{-1}$) and dwell time 1 msec were set.

2.6. GC–MS/MS instrumentation

GC–MS/MS (TQ-8039, Shimadzu®, Kyoto, Japan) was used to separate and quantify 34 pesticides in okra fruits. The gas chromatograph is equipped with MS triple quadrupole coupled with an AOC-20i injector and AOC-20 s auto-sampler. The GC–MS/MS is controlled using Labsolution® software (Version 1.5). The separations of target pesticides were performed using a capillary fused silica HP 5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with splitless injection (2 μL) mode. The oven temperature program was as follows; the initial temperature was set at 60 °C for 2 min, ramped at the rate of 25 °C min^{-1} to 150 °C, 3 °C min^{-1} rate to 200 °C, 8 °C min^{-1} to 280 °C for 5 min. and finally ramped to 300 °C at 25 °C min^{-1} for 3 min. All the pesticides clearly separated within the total run time of 25 min. The carrier gas was helium (99.999% purity), with a constant flow rate of 1.5 mL min^{-1}, surge pressure of 250 kpa, and injector temperature 280 °C was maintained.

MS parameters were as follows; transfer line and ion source temperature was set at 280 and 250 °C, respectively. Ionization was done through positive electron impact (EI) at –70 eV. The MRM optimization was performed by obtaining the product ion scan and collision energy for each transition. The solvent delay was fixed at 3 min. Argon (Ar) and helium (He) with a flow rate of 1.50 and 2.25 mL min^{-1} were maintained as collision and quench gases. The mass range was between 50 and 550 *m/z*.

2.7. Method validation

The European Commission document SANTE/12682/2021 was followed to validate the developed method with LC-MS/MS and GC–MS/MS. The criteria *viz.,* linearity, matrix effect, limit of quantification (LOQ), trueness (recovery), precision (intraday), precision (interday), ion ratio, and retention time were assessed for 73 pesticides in the okra matrix.

2.7.1. Sensitivity/linearity

A linearity study was performed, preparing different concentrations of pesticide mixture ranging from 0.01 to 1.0 μg mL^{-1} in solvent standard and matrix extract of okra fruit. Estimated the response (residuals) of linear concentration after injection into the instruments based on the linear regression equation. The linearity graphs were drawn by plotting concentrations against the response and recording the coefficient of determination (R^2). Residuals were calculated using the following formula:

$$\text{Deviation of back-calculated concentration (\%)} = (C_{measured} - C_{true}) \times 100/C_{true}$$

$$\text{Measured concentration } \left(ng\,g^{-1}\right) = \frac{\text{Area of sample} - \text{Intercept (c) of linearity}}{\text{Slope (m) of linearity}}$$

C_{true}– Linearity standard concentration (ngg^{-1}).

2.7.2. Matrix effect (ME)

The complex matrices were responsible for suppressing and enhancing analyte signals or responses in LC-MS/MS and GC–MS/MS. It was evaluated using the following formula.

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

where bm and bs are the angular coefficient from the matrix and the solvent linearity studies, respectively (Silva, Habermann, Marchi, & Zocolo, 2012; Naik et al., 2021).

2.7.3. Limit of quantification

The LOQ is the lowest spiked concentration that could be quantified and recorded acceptable recovery in the range of 70–120% and relative standard deviation of ≤ 20%. Further, the lowest concentration level for the instruments such as LC-MS/MS and GC–MS/MS could quantify the residue less than MRLs of regulatory bodies.

2.7.4. Trueness (Recovery)

The trueness or recovery was conducted spiking pesticide standard mixture at 10, 50, and 100 μg kg^{-1} to the blank okra samples and extracted following the procedure standardized. Matrix matched calibration standards responses were used to compare the responses recorded in the spiked samples and quantify the residues and calculated percent recovery.

2.7.5. Precision (intra and inter-day)

Intraday and interday precision (Repeatability-RSDr and Reproducibility-RSDwr) was assessed separately at 50 μg kg^{-1} spiking level. Intraday precision was performed in six blank extract samples using the standardized method and recorded the LC-MS/MS and GC–MS/MS response. Similarly, the interday precision test was conducted on two different days and calculated the recovery and RSD.

2.7.6. Ion ratio

For MS/MS triple quadrupole, a minimum of 2 product ions is essential as per the SANTE guidelines. This method selected two product ions and a precursor ion for each pesticide compound after injecting individual pesticides to LC-MS/MS and GC–MS/MS. The ion ratio was calculated based on the peak area or intensity ratio of low intense ions to that high intense ions. The ion ratio value was calculated as,

$$\text{Ion ratio} = \frac{\text{Peak area (confirmation ion)}}{\text{Peak area (quantitation ion)}} \times 100$$

The ion ratio from sample extracts was estimated, and ions recorded less than or equal to ± 30% (relative) were considered for confirmation of the analytes.

2.7.7. Retention time (RT)

The good retention time of target analytes in the LC-MS/MS and GC–MS/MS chromatogram was examined from the calibration standard and matrix match standard solutions with a tolerance limit of ± 0.1 min.

2.8. Estimation of measurement uncertainty

Uncertainty due to repeatability test from the intra-laboratory validation process (also called type 'A' uncertainty: U), combined uncertainty (Uc), and expanded uncertainty (Uexp) was estimated for all the insecticides following EURACHEM/CITAC Guide (Ellison & Williams, 2012; NABL 164, 2016; Naik et al., 2021; Harischandra et al., 2021). The mathematical formulae for estimation of uncertainty (U_{exp} at 95% confidence limit) are given below.

Uncertainty due to repeatability

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

Combined Uncertainty (Type 'B' Uncertainty),

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

Coverage factor (k) at 95 % Confidence level

$$V_{effective} = \frac{(U_C)^4}{\frac{(U_1)^4}{(d.f)} + \frac{(U_2)^4}{(d.f)} + \cdots + \frac{(U_n)^4}{(d.f)}} \quad (3)$$

Where, U_C is the Combined Uncertainty, U_1, U_2, …U_n are relative uncertainty of individual component and d.f is degrees of freedom for each component.

Expanded Uncertainty (at 95% confidence; $k = 2$) is estimated as

$$U_{exp} = U_c \times k; \quad (4)$$

Where, U_{exp} is Expanded Uncertainty; k is the coverage factor (k) at 95% Confidence level and U_C is the Combined Uncertainty.

2.9. Quality assurance

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. Further, 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}).

2.10. Screening of unknown samples and risk assessment

Twenty-five okra fruit samples from the field and market were collected and screened for the pesticide residues following validated methods. The samples were extracted and clean-up with the standardized protocol and quantified the residues. Further, the highest residues for detected pesticides were considered to assess the risk of following the Hazard Index (HI) model. Initially, the estimated daily intake (EDI) was calculated by multiplying the residues of individual pesticides with per capita okra fruits (vegetables as whole) consumption rate (kg day^{-1}) by the adult and children. Then, the hazard index was estimated by dividing EADI of individual pesticides through their corresponding ADI (Acceptable Daily Intake). The safety of the okra fruits collected from the market and fields was categorized based on hazard index values. It is presumed that if HI is>1, then vegetable food is not safe for consumption (Darko & Akoto, 2008; Kumari and Jhon, 2019).

3. Results

3.1. Optimization of LC-MS/MS parameters

The optimized liquid chromatographic conditions with different mobile phases (A and B) utilized to separate the pesticides provided proper ionization. The stationary phase of the C_{18} column (octadecylsilyl, 2 mm i.d × 150 mm × 0.2 μm) separated and recorded good peak shape for all the 39 pesticides with a gradient LC time program of 25 min in LC-MS/MS (Fig. 1). The most abundant *m/z* ions (mass-to-charge) for each pesticide were recorded through full scan mass spectrums by injecting 2 μL of individual pesticide standards. The protonated molecular ion $(M + H)^+$ was determined and recorded as precursor ion (Table S1). Further, 2 μL of 0.1 μg mL^{-1} pesticide standard mixture was injected into the LC-MS/MS to optimize the MRM (multiple reaction monitoring) transitions and acquisition for a higher abundance of the fragmented ions through positive electrospray ionization (ESI +). Finally, dissociation with argon gas was induced, and the different collision energy was tested to record the most abundant productions. Three different ions *viz.,* a precursor, and two product ions used to quantify and confirm the target pesticide in the samples. The selected MRM transitions recorded higher sensitivity and selectivity for the pesticides at 0.01 to 1.00 mg kg^{-1} in the okra fruit matrix (Table S1).

3.2. Optimization of GC–MS/MS

The targets of 34 pesticides were determined in a single GC run with trifluralin compound was first detected with the retention time of 12.34 min, and last deltamethrin compound was detected with the retention

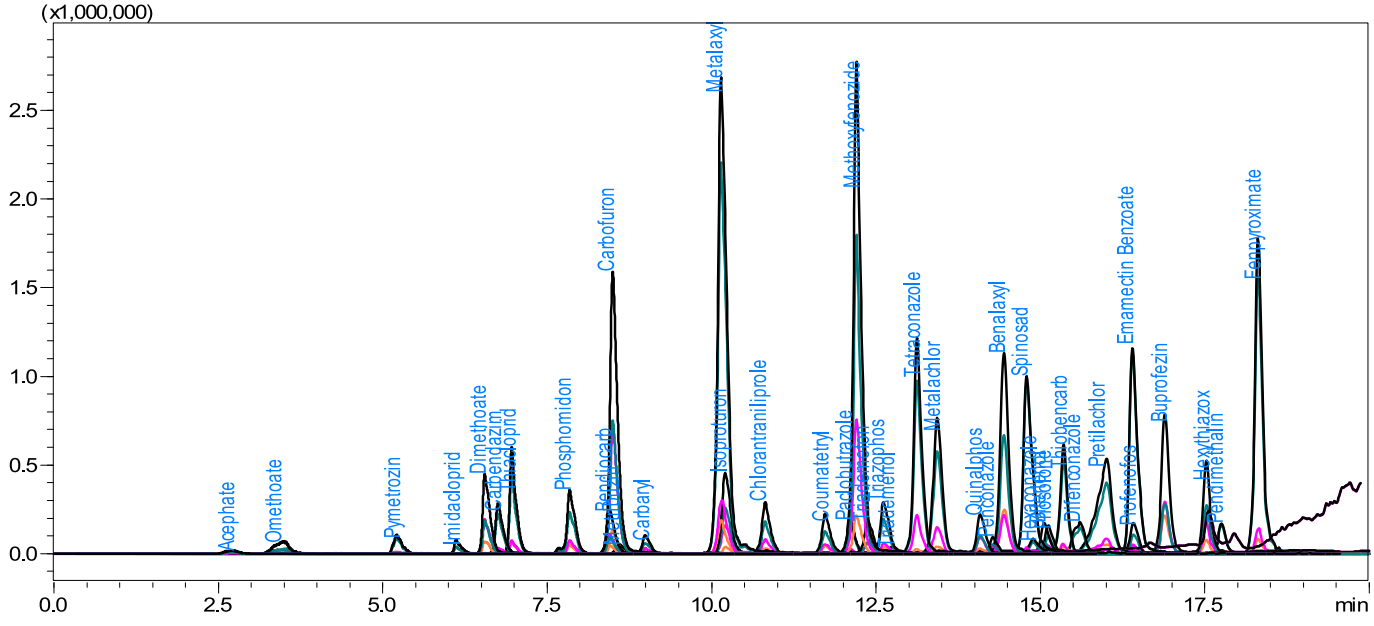

Fig. 1. MRM Chromatogram of 39 LC pesticides recovery in okra matrix fortified at 0.01 $mgkg^{-1}$.

time of 34.64 min (Fig. 2). All the target pesticides were eluted with good resolution and a retention time deviation of ± 0.1 min. The GC–MS/MS parameters were optimized to detect, confirm, and quantify the residue of selected pesticide residues in okra fruit. The mass spectra obtained from the full scan or total ion chromatogram of target compounds showed the most abundant ion at *m/z* (100% relative abundance) was selected as precursor ion and productions selected for confirmation of the pesticides in the okra fruit matrix. Further, product ions scan was performed with different Collision Energies (CE) to increase the sensitivity of target compounds. For each pesticide, multiple reaction monitoring (MRM) transitions with appropriate CEs were determined (Table S1) and further simplified by using the smart MRM optimization tool (SANTE/12682/2019).

3.3. Method validation

The method optimized 73 pesticide residues in okra fruit using LC-MS/MS and GC–MS/MS (Table 1 & 2). All the pesticides were determined in a single run time of 25 and 41.07 min in LC-MS/MS and GC–MS/MS, respectively, with the coefficients of determination ($R^2 >$ 0.998) with the linear concentration of 0.01 to 1.00 mg kg^{-1}. The LOQ of all the pesticides in okra fruits was considered 10 μg kg^{-1} for both GC and LC techniques due to the acceptable recovery recorded with this proposed method. This level is less than MRLs (EU and FSSAI) of pesticides considered in this study.

The analytical method was found to be efficient for the okra fruit. The obtained recoveries were ranged between 70 and 120%. Okra fruit was spiked at 10, 50, and 100 μg kg^{-1} level recorded recovery in the range of 72.23 to 116.83%, 71.94 to 109.06%, and 73.94 to 116.57%, respectively, and RSD at different fortification levels were found less than 15% for LC-MS/MS (Table 1 & Fig. 2). For GC compatible pesticides, recovery was in the range of 72.14 to 119.70 %, 70.03 to 88.91%, and 75.07 to 115.72% with relative standard deviation less than 15% at 10, 50, and 100 μg kg^{-1} levels, respectively (Table 2 & Fig. 5). Similar recoveries (<90%) were observed for okra at 25 μg kg^{-1} (Banerjee et al., 2012). The recovery in both techniques was found acceptable as per the validation guidelines.

Intraday precision estimation at 50 μg kg^{-1} recorded the recovery range of 70.69 to 106.36%, with RSD less than 12.86%. The precision with inter-day test recorded the recovery of 73.31 to 104.23 % with RSD less than 15% for pesticide tested in LC-MS/MS. The intraday precision test recorded 72.31 to 117.86 % recovery, and inter-day precision recorded 74.14 to 114.97% recovery and RSD less than 15% for the GC–MS/MS. Overall, the present study on method validation with okra fruit matrix fulfilled the SANTE/12682/2019 guidelines. The ratio between quantifier and qualifier ions was used as a confirmatory tool for the pesticides in the okra fruit matrix. The ion ratio of both LC-MS/MS and GC–MS/MS methods was less than 30%. With the chromatographic conditions set in the present methods, selected pesticides were separated, and retention time of all pesticides on okra fruit in GC–MS/MS and LC-MS/MS were found to be within the acceptable limit of ± 0.1 min.

3.4. Matrix effect

Matrix effect is common in different food matrices, and matrix match calibration standards are routinely used to nullify the matrix effect in the chromatographic method. It was assessed using the blank extracts (without target pesticides) of okra fruit for matrix match calibration. Most of the selected pesticides showed an acceptable matrix effect within the range of −2.44 to 37.24 %. The matrix effect observed in the present method for okra fruit sample was considered to be less to moderate matrix effects as per Ferrer, Lozano, Agüera, Jiménez, and Fernández (2011), who classified the matrix effects into three different categories *viz.,* less (<20%), moderate (20–50%) and strong (>50%) based on signal suppression and or enhancement. The calculated matrix effect was much below the maximum threshold limit of 20% signal suppression or enhancement. For more realistic estimation and quantification of pesticide residues in the samples, the response of matrix-matched calibration standard is suggested to compare with pesticide response in the unknown samples, and quantified pesticide concentration would be the actual value (Silva et al., 2012; Naik et al., 2021 & Harischandra et al., 2021). Overall, the matrix effect with both techniques was significant. Comparing the matrix effects observed in grape, mango, capsicum, okra, and drumstick for multi-class pesticides was moderate to low matrix effect (Jadhav et al., 2015). The present study

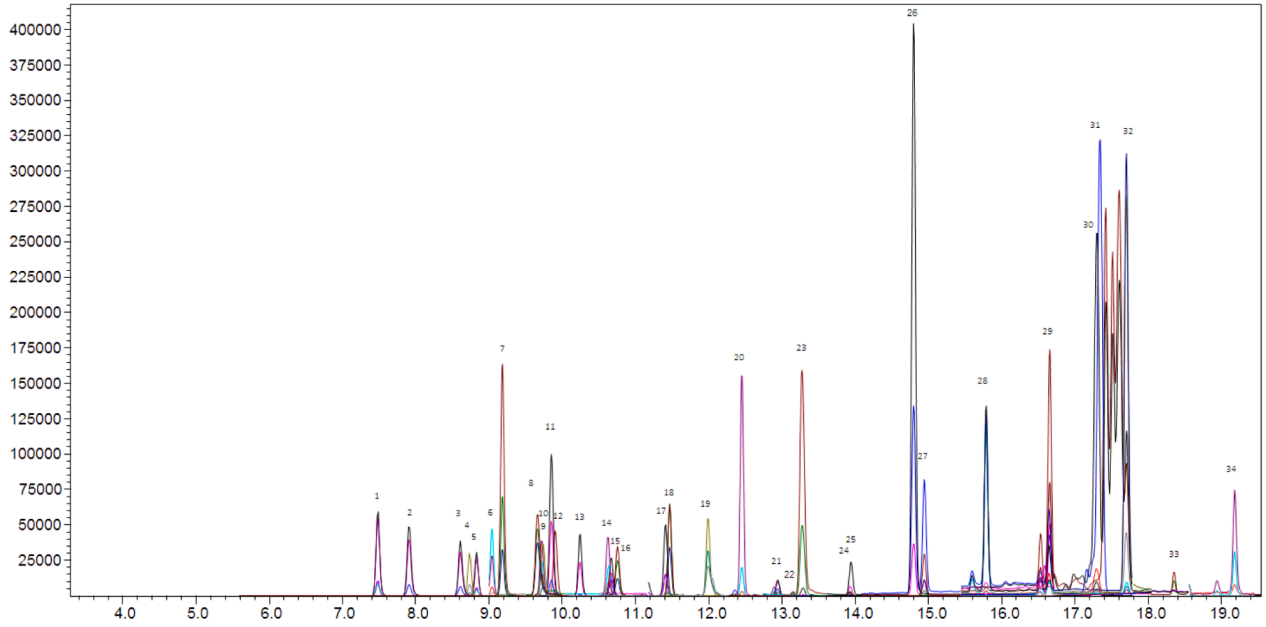

Fig. 2. GC–MS/MS MRM chromatogram for standard concentration at 10 μgkg^{-1}.

Table 1
Recovery, precision (intra and inter day), matrix effect, EU- MRL and uncertainty for pesticides in okra fruits using LC-MS/MS.

Pesticide	Recovery % (RSD %)			Intra-day precision (50 μg kg^{-1})		Inter-day precision (50 μg kg^{-1})		Matrix effect (%)	EU MRL (mg/kg)	U_c at 50 μgkg^{-1}
	10 μg kg^{-1}	50 μg kg^{-1}	100 μg kg^{-1}	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)			
Thiacloprid	101.7 (8.78)	104.50 (10.48)	101.24 (8.30)	98.67	9.29	102.23	10.09	8.19	0.01	12.48
Buprofezin	88.55 (10.32)	95.80 (9.17)	101.55 (8.66)	93.67	9.10	94.36	12.63	12.74	0.01	8.51
Metalachlor	98.80 (8.12)	109.06 (10.53)	98.62 (10.50)	96.68	9.61	92.31	14.89	10.79	0.05	10.50
Imidacloprid	96.21 (10.35)	97.36 (8.69)	92.30 (9.91)	92.38	11.05	98.31	6.41	23.19	0.50	12.48
Dimethoate	101.41 (7.27)	94.89 (7.34)	110.96 (8.60)	96.36	9.98	95.26	13.28	27.24	0.01	9.97
Coumatetryl	81.90 (8.26)	89.18 (8.72)	82.11 (10.30)	84.28	9.90	85.21	5.58	24.14	–	9.97
Triadimenol	73.05 (11.94)	78.98 (10.39)	77.19 (9.77)	76.36	10.39	76.16	6.09	16.53	0.01	10.21
Triadimefon	99.63(8.49)	100.29 (9.18)	116.57 (10.35)	99.86	9.87	98.31	5.30	32.43	0.01	11.62
Thiobencarb	86.35 (13.36)	82.14 (11.69)	82.56 (9.60)	86.36	12.86	87.31	4.31	−3.59	0.01	11.63
Spinosad	111.56 (9.79)	93.19 (6.13)	80.52 (10.03)	94.35	10.10	96.28	5.51	1.96	0.02	12.57
Phosalone	112.05 (7.52)	110.59 (8.52)	102.99 (10.02)	106.36	12.54	104.21	12.51	7.52	0.01	10.25
Methoxyfenozide	74.37 (11.03)	71.94 (7.82)	73.94 (9.82)	70.69	9.98	73.31	10.33	15.90	0.01	9.80
Hexythiazox	102.78 (13.57)	104.49 (9.26)	102.29 (9.71)	100.26	12.50	104.23	13.57	18.24	0.50	11.99
Fenpyroximate	88.54 (11.63)	92.02 (10.65)	92.73 (9.48)	90.19	11.24	93.21	11.22	15.78	0.01	12.05
Carbendazim	104.42 (9.56)	93.30 (9.25)	95.53 (9.01)	94.86	11.05	95.07	9.18	28.10	2.00	11.29
Carbaryl	89.53(8.42)	90.72 (8.57)	101.61 (8.41)	91.23	10.59	94.26	6.63	10.74	0.01	9.07
Triazophos	95.68(7.54)	84.15 (10.57)	90.83 (10.26)	83.18	10.53	86.26	14.55	19.80	0.01	10.87
Carbofuron	77.72 (8.98)	82.28 (7.51)	85.08 (9.52)	86.68	9.31	85.21	10.02	12.07	0.002	8.98
Bitertanol	103.76 (8.80)	100.03 (9.67)	98.90 (8.26)	97.68	10.86	96.28	9.57	14.46	0.01	12.26
Bendiocarb	89.51(6.52)	87.04 (10.55)	91.09 (8.05)	88.25	12.08	84.23	12.85	20.37	–	10.04
Benalaxyl	116.81 (9.39)	94.69 (9.04)	92.56 (8.94)	94.36	11.88	98.23	12.25	13.63	0.05	9.68
Acephate	76.63(6.06)	83.54 (10.19)	84.81 (9.54)	87.86	12.19	89.26	5.86	27.21	0.01	10.71
Pymetrozine	81.69 (12.22)	81.23 (12.25)	87.10 (9.33)	84.29	10.66	88.13	7.99	9.80	0.01	11.66
Omethoate	88.92(9.84)	82.94 (10.01)	86.15 (9.94)	81.69	12.65	83.23	10.30	10.34	0.01	12.84
Metribuzin	73.87 (11.32)	78.83 (12.50)	84.32 (9.84)	74.39	12.60	81.06	12.92	21.56	0.1	9.97
Metalaxyl	109.25 (9.43)	100.38 (8.76)	101.27 (9.72)	101.29	10.42	103.26	9.38	−4.38	0.01	9.57
Emamectin Benzoate	113.69 (6.65)	103. 61 (9.69)	99.47 (8.03)	100.17	11.05	99.86	8.10	4.93	0.02	8.35
Tetraconazole	106.24 (6.18)	90.06 (7.12)	92.78 (9.23)	94.26	10.20	96.16	13.13	−11.05	0.02	9.65
Quinalphos	91.61(8.55)	101.40 (1132)	101.79 (8.83)	102.30	9.95	100.39	8.80	20.97	0.01	12.19
Profenofos	72.23 (14.53)	78.77 (8.09)	83.26 (9.82)	74.26	11.36	74.19	8.32	25.58	0.01	12.30
Phosphomidan	99.18 (11.32)	106.13 (9.79)	95.38 (10.36)	102.36	12.62	104.23	12.68	−9.04	0.01	9.87
Pendimethalin	95.60 (12.01)	100.06 (11.42)	102.77 (9.41)	96.29	12.38	98.38	9.48	13.51	0.05	10.03
Difenconazole	96.76 (9.30)	97.56 (10.35)	101.44 (10.21)	99.16	8.90	100.01	10.68	−6.93	0.06	9.96
Pretilachlor	106.81 (8.88)	95.17 (8.65)	95.77 (9.50)	102.36	10.42	97.62	12.18	20.00	–	10.96
Paclobutrazole	105.30 (9.68)	91.98 (10.74)	99.22 (8.77)	92.38	9.45	90.26	14.99	11.51	0.01	12.91
Chlorantraniliprole	111.91 (7.06)	100.04 (11.69)	96.89 (10.43)	103.68	12.83	103.00	5.44	2.54	0.60	11.63

(*continued on next page*)

Table 1 (*continued*)

Pesticide	Recovery % (RSD %)			Intra-day precision (50 μg kg^{-1})		Inter-day precision (50 μg kg^{-1})		Matrix effect (%)	EU MRL (mg/kg)	U_c at 50 μgkg^{-1}
	10 μg kg^{-1}	50 μg kg^{-1}	100 μg kg^{-1}	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)			
Isoproturon	104.83 (7.99)	93.20 (9.76)	97.95 (10.37)	94.28	12.21	96.68	8.82	15.59	0.01	10.50
Hexaconazole	84.23 (7.87)	88.59 (11.52)	81.10 (8.71)	86.35	9.89	87.23	11.25	23.84	0.01	8.40
Penconazole	98.51 (12.38)	105.09 (8.36)	102.40 (9.56)	101.34	9.82	96.25	14.81	8.00	0.01	10.05

MRL-maximum residue limit; EU-European Union; U_c-Combined Uncertainty.

Table 2
Recovery, precision (intra and inter day), matrix effect, EU-MRL and uncertainty for pesticides in okra fruits using GC–MS/MS.

Pesticide	Recovery % (RSD %)			Recovery (RSD %) @ 50 μgkg^{-1}		Matrix effect (%)	EU MRL (mg/kg)	U_c at 50 μgkg^{-1}
	10 μgkg^{-1}	50 μgkg^{-1}	100 μgkg^{-1}	Intra-day	Inter-day			
Fluchloralin	94.77 (14.87)	77.10 (12.07)	76.06 (5.22)	91.03 (14.92)	91.02 (17.78)	12.65	–	2.69
alpha-BHC	79.53 (14.48)	79.15 (10.66)	77.83 (8.79)	78.52 (10.57)	80.70 (10.03)	6.71	0.01	3.38
beta-BHC	89.51 (13.53)	72.95 (9.10)	83.17 (7.80)	88.57 (14.99)	86.75 (14.06)	6.40	0.01	2.43
Diazinon	113.15 (9.43)	70.78 (14.12)	79.78 (13.05)	93.57 (16.66)	97.33 (16.12)	6.86	0.01	3.32
Trifluralin	89.83 (13.39)	79.06 (12.14)	78.03 (7.27)	90.01 (15.89)	91.87 (11.72)	20.58	0.01	10.70
Tri-allate	102.06 (12.70)	77.88 (10.98)	75.95 (12.10)	84.12 (16.88)	84.90 (16.57)	6.80	0.1	6.38
Iprobenfos	108.10 (9.76)	83.24 (10.02)	98.38 (8.30)	106.59 (11.74)	111.29 (10.01)	15.57	–	3.97
Propanil	117.76 (11.26)	78.32 (11.41)	90.11 (6.67)	117.86 (11.79)	105.73 (12.11)	5.00	0.01	7.67
Chlorpyrifos-methyl	115.57 (15.26)	73.39 (14.29)	82.51 (6.26)	83.64 (17.59)	92.08 (17.59)	12.73	0.01	2.28
Parathion-methyl	76.39 (15.51)	74.88 (7.58)	91.51 (7.47)	96.60 (12.34)	93.17 (9.98)	14.01	0.01	3.58
Alachlor	118.00 (9.14	83.76 (9.71)	97.66 (8.66)	106.00 (10.83)	110.44 (9.91)	8.96	0.01	1.81
Heptachlor	91.98 (12.65)	77.14 (14.3)1	75.07 (12.07)	73.46 (15.16)	75.54 (16.82)	13.88	0.01	1.93
Fenitrothion	116.34 (4.58)	70.77 (10.22)	87.10 (7.42)	95.27 (8.79)	102.22 (16.74)	14.29	0.01	6.08
Aldrin	98.58 (10.79)	71.97 (9.54)	76.58 (8.63)	74.69 (15.72)	72.31 (14.66)	4.12	0.02	9.02
Chlorpyrifos	112.04 (11.51)	88.91 (15.59)	79.27 (5.35)	80.50 (16.13)	87.28 (16.28)	6.40	0.01	2.38
Parathion	75.36 (12.30)	76.66 (7.38)	82.68 (6.07)	91.81 (13.96)	96.23 (9.01)	5.92	0.05	3.18
Chlorofenvinphos	117.22 (8.59)	74.88 (12.68)	92.13 (8.34)	97.53 (15.97)	107.82 (14.11)	37.24	0.01	6.36
Phenthoate	83.23 (10.71)	84.47 (7.89)	86.68 (8.42)	108.55 (16.23)	110.96 (13.17)	13.54	–	1.96
Butachlor	93.81 (11.25)	74.47 (14.85)	99.44 (9.66)	95.81 (11.09)	101.10 (10.05)	19.20	–	3.63
p,p'-DDE	76.66 (7.31)	75.31 (9.74)	79.91 (3.66)	83.08 (11.36)	78.62 (13.53)	3.95	0.05	3.21
Endrin	79.83 (12.41)	71.77 (13.48)	84.12 (7.24)	79.76 (11.45)	86.23 (19.22)	−5.39	0.01	10.11
beta-Endosulfan	119.70 (14.82)	75.97 (13.18)	79.38 (13.06)	116.16 (30.99)	98.73 (28.26)	−8.70	0.05	11.27
o,p'-DDT	87.41 (10.73)	70.68 (8.85)	88.80 (3.38)	92.52 (13.28)	88.01 (10.71)	−3.36	0.05	4.67
Endosulfan sulfate	86.70 (11.75)	76.02 (15.15)	115.72 (14.41)	93.56 (44.20)	93.73 (29.05)	1.00	0.05	2.78
p,p'-DDT	72.14 (11.61)	84.36 (14.16)	77.40 (12.35)	77.10 (16.12)	75.29 (18.33)	−2.44	0.05	5.69
Bifenthrin	99.98 (12.14)	82.23 (6.90)	101.08 (7.26)	99.16 (10.37)	99.05 (9.80)	−18.18	0.20	6.17
Fenpropathrin	100.20 (12.45)	81.93 (9.03)	108.23 (10.14)	106.85 (11.30)	114.97 (10.07)	−9.40	0.01	11.99
lambda-Cyhalothrin	78.42 (12.30)	86.09 (5.63)	103.51 (6.87)	111.77 (8.67)	114.91 (7.29)	3.15	0.30	9.36
Permethrin	72.60 (12.70)	78.47 (6.46)	81.93 (4.00)	100.58 (7.58)	87.31 (10.22)	0.77	0.05	6.30
Cyfluthrin	79.53 (13.14)	77.95 (7.62)	80.23 (2.70)	95.62 (8.33)	93.82 (8.34)	−6.50	0.02	12.16
Cypermethrin	73.24 (15.03)	70.25 (6.02)	83.97 (3.78)	95.14 (8.17)	92.25 (7.92)	−7.96	0.50	12.39
Etofenprox	78.79 (12.10)	79.78 (14.95)	77.83 (13.45)	79.73 (14.78)	76.22 (14.09)	−6.59	0.01	12.27
Fenvalerate	77.91 (13.86)	74.65 (12.32)	79.91 (9.42)	96.94 (15.92)	72.09 (18.98)	−9.72	0.02	11.16
Deltamethrin	70.08 (14.21)	70.03 (10.20)	87.94 (5.10)	83.46 (9.66)	85.66 (7.21)	−9.58	0.01	9.54

MRL-maximum residue limit; EU-European Union; U_c-Combined Uncertainty.

recorded a similar observation with okra matrix for 73 pesticides with GC–MS/MS and LC-MS/MS.

3.5. Estimation of measurement of uncertainty

Uncertainty due to repeatability was estimated for a minimum of 6 different consecutive extraction of the okra fruit samples spiked at 50 μg kg^{-1} produced acceptable recoveries and could contribute significantly to the measurement uncertainty. The uncertainty values were ranged from 1.81 to 12.91 μg kg^{-1}estimated at 50 μg kg^{-1}. Combined uncertainty is the inclusion of all the relative uncertainty of individual component *viz.,* sample weight, weighing of standards, preparation stock (1000 μg mL^{-1}), intermediate (100 μg mL^{-1}) and working solution (10 μg mL^{-1} and 0.5 μg mL^{-1}), use of micropipette (100 μL) for linearity, calibration curve, recovery of analyte and certified reference material. Combined uncertainty is a criterion for deciding on the sample results when a quantified analyte is reported value near or equal to the LOQ and MRLs of that particular contaminant. In such cases, the combined uncertainty is considered a tool to decide analytes' fate, whether above or below MRLs, and declare the results (NABL, 164). In the present study, 73 multi-class pesticides recorded acceptable uncertainty measurement values (Table 1 &2). Similar results were reported previously found in made tea and tea infusion and spent leaves (Kanrar, Mandal, & Bhattacharyya, 2010; Banerjee et al., 2007); the uncertainty of measurement for aflatoxin maize and fig (Stadler, Sulyok, Schuhmacher, Berthiller, & Krska, 2018), pigeonpea and rice grain (Naik et al., 2021; Harischandra et al., 2021).

3.6. Real samples analysis and risk assessment

A total of 25 okra fruit samples were screened separately for both LC-MS/MS and GC–MS.MS. The fenpyroxymate, carbendazim, profenofos, acephate, imidacloprid, hexaconazole, emamectin benzoate, triademenol and bifenthrin was detected in the farm field and market okra

fruit samples analyzed by LC-MS/MS and GC–MS/MS at residue concentrations of 0.01 to 0.11 mg kg^{-1} and 0.01 to 0.16 mg kg^{-1}, respectively for field and market samples (Table 3 & 4; Fig. 3 & 6). Further, the data subject to the risk assessment revealed that the Hazard Index value for the maximum residues for profenofos was>1. It is having the risk to both the consumer category (children and adults). Whereas the insecticides detected in the market samples did not have risk except the profenofos. None of the other insecticides has significant toxicological risk in this study, and that would not cause any hazardous to the consumers.

4. Discussion

4.1. Method comparison

The present investigation reported a highly sensitive and reproducible analytical method with LC-MS/MS and GC–MS/MS to simultaneously determine 73 pesticide residues in okra. The method is short-run and reproducible; meeting different regulatory body requirements, FSSAI, APEDA, EU and Codex Alimentarius Commission tolerance limits (MRLs less than 10 μgkg^{-1} for most of the pesticides). The method had high accuracy with acceptable recovery at 0.01 mg kg^{-1} (as quantification limit), less than or equal to EU-MRL. Compared to the previously published method on okra and other vegetable matrices, the present investigation has significant findings with respect to recovery, inter and intraday precision, matrix effect and simultaneous determination of chemical pesticides. To support the claim, Kumari et al. (2005) reported a method with GC-ECD and NPD to analyze organochlorine, organophosphate, synthetic pyrethroids, and carbamates residues in vegetables with limits of detection in the range of 5–100 picograms and this method lacks the confirmation of pesticides residues in vegetables. Baig, Akhtera, Ashfaq, and Asi (2009) reported a method for eggplant plants, pumpkins, and okra using HPLC with a UV detector. This method is having less sensitive, and the technique used is non-confirmatory. Analysis of multi-residues using HPLC or GC in different vegetable foods could be less accurate and acceptability at global food trade for export. The tandem mass spectrometry is otherwise an essential tool for quantifying residues in vegetable foods.

Pesticide residue determination in okra fruits with an analytical method using GC–MS found 70 to 120% of recovery with 1:1n-hexane and dichloromethane extraction (liquid–liquid extraction) with florisil clean-up column of okra fruits at 0.10 μg kg^{-1} fortification (Essumang, Asare, & Dodoo, 2013). To quantify pyrethroid residues, the method developed using gas chromatography electron capture detector by Chandra (2008) and Kumari et al. (2005) inferior to the method developed on mass spectrometry. Saeid and Selim (2012) standardized a method to estimate the 86 different crop protection pesticides using GC–MS with a detection limit of 0.01mgkg^{-1} for raw non-leafy vegetables. This method reported a high sample size of test portion (120 g) and extraction solvent (200 mL) acetonitrile is absolutely a non-economical approach. However, it involves a mass technique for the detection and quantification of multi-group pesticides.

A method using LC-MS/MS was reported to determine fipronil and its metabolites simultaneously, and difenoconazole in okra provided 80–107% recoveries with an RSD range between 4 and 17% LOQ ranged 1 to 5 ng g^{-1} (Hingmire et al., 2015). However, Banerjee et al. (2012) reported a multi-residue method for different fruits and vegetables, including okra using GC-EI-MS/MS with LOQ of less than 10 μg/L and acceptable recovery range of 70–110% and RSD less than 20% is a similar method concerning proposed method in the present investigation. Whereas, Jadhav et al. (2015) reported a similar method for pesticides in fruits and vegetables with 70–120% recoveries and RSD less than 20% are the latest methods available. Present methods are superior to those published previously in terms of sensitivity, accuracy, repeatability, and reproducibility. A significant part in these methods is confirmation of the residues in the market and field collected samples. An analytical method needs to ensure the analyte detection in the market and field samples to judge the quality of the vegetable foods and other agricultural products. Further, it would support the global food standard requirements.

4.2. Residues in real samples from farm-gate and markets

In the present investigation, 73 agrochemicals were screened in okra fruit for the presence of residue using tandem mass spectrometry. After analyzing the field samples, the residues of fenpyroxymate (0.01 mg kg^{-1}), carbendazim (0.11 mg kg^{-1}), profenofos (0.02 mg kg^{-1}), acephate (0.11 mg kg^{-1}), imidacloprid (0.02 mg kg^{-1}), hexaconazole (0.03 mg kg^{-1}), emamectin benzoate (0.01 mg kg^{-1}), triademenol (0.01 mg kg^{-1}) and bifenthrin (0.02 mg kg^{-1}) were quantified and confirmed simultaneously. There is no much difference for the residues observed in the market sample. Similar compounds were quantified from the market okra samples; however, the residue level was higher than in field samples (Table 3 & 4).

Multiresidue analysis in market samples across the globe could reveal the significant amount of residues of pesticides. A real sample of okra from the farm gate market outlets showed substantial residues when analyzed with the proposed method. However, during 2003, about 20 different market okra samples were analyzed for OC, OP, SP, and carbamates insecticides using a primitive analytical tool *viz.*, GC-ECD and NPD reflected the residues of monocrotophos, malathion, endosulfan, and cypermethrin. This study reported the pesticide contamination of market samples, residues of monocrotophos, and cypermethrin found above their tolerance level (MRL) (Kumari & John, 2005). Analysis of organophosphate pesticide residues in 36 okra samples collected from different farms in Pakistan showed profenophos, triazophos, and chlorpyriphos in 7, 5, and 1 sample, respectively exceeded the prescribed MRLs (Baig et al., 2009). Another study involving analysis of 350 vegetables (okra, cabbage, tomato, lettuce, carrot, green pepper, onion, and cucumber) samples collected from six different markets located at Kumasi (Ghana) found to be 19 and 43.5% of the samples had residues above and below the MRL, respectively (Crentsil, Archibold, Dzifa,

Table 3
Health risk estimation in okra fruits collected at from Field.

Pesticide	Residue (mg kg^{-1})	EDI (mg kg^{-1} body weight)		ADI (mg kg^{-1} body weight)	Hazard Index	
		Children	Adult		Children	Adult
Fenpyroxymate	0.01	0.00442	0.00074	0.01	0.10	0.02
Carbendazim	0.11	0.00071	0.00012	0.03	0.15	0.02
Profenofos	0.02	0.00425	0.00071	0.00	7.13	1.19
Acephate	0.11	0.00071	0.00012	0.03	0.14	0.02
Imidacloprid	0.02	0.00135	0.00022	0.06	0.01	0.00
Hexaconazole	0.03	0.00058	0.00010	0.01	0.27	0.04
Emamectin benzoate	0.01	0.00059	0.00010	0.00	0.29	0.05
Triademenol	0.01	0.00093	0.00015	0.06	0.01	0.00
Bifenthrin	0.02	0.00442	0.00074	0.01	0.09	0.02

EDI-estimated daily intake, ADI-acceptable daily intake,

Table 4
Health risk estimation in okra fruits collected from the market.

Pesticide	Residue (mg kg^{-1})	EDI (mg kg^{-1} body weight)		ADI (mg kg^{-1} body weight)	Hazard Index	
		Children	Adult		Children	Adult
Metalachlor	0.01	0.00026	0.00004	0.20	0.00	0.00
Carbendazim	0.16	0.00621	0.00103	0.03	0.21	0.03
Profenofos	0.01	0.00040	0.00007	0.00	4.00	0.67
Bifenthrin	0.01	0.00053	0.00009	0.01	0.05	0.01
Acephate	0.04	0.00147	0.00025	0.03	0.05	0.01
Imidacloprid	0.09	0.00349	0.00058	0.06	0.06	0.01
Hexaconazole	0.12	0.00492	0.00082	0.01	0.98	0.16
Emamectin benzoate	0.02	0.00087	0.00014	0.00	0.43	0.07
Fenpyroxymate	0.01	0.00058	0.00010	0.01	0.12	0.02

EDI-estimated daily intake, ADI-acceptable daily intake.

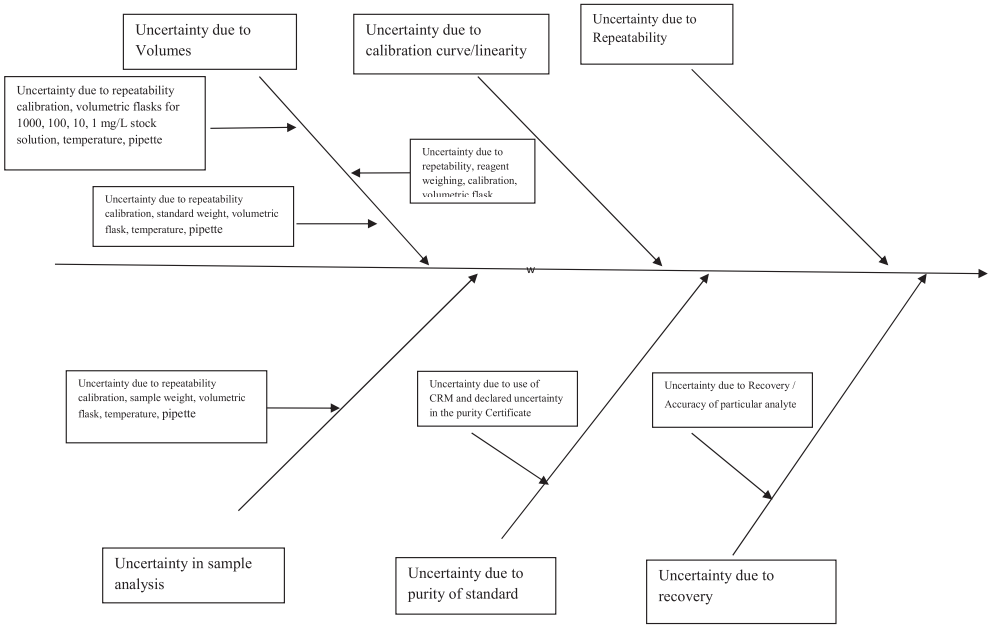

Fig. 3. Ishikawa diagram presenting the uncertainty sources of the measurement uncertainty for pesticide in okra samples analysis.

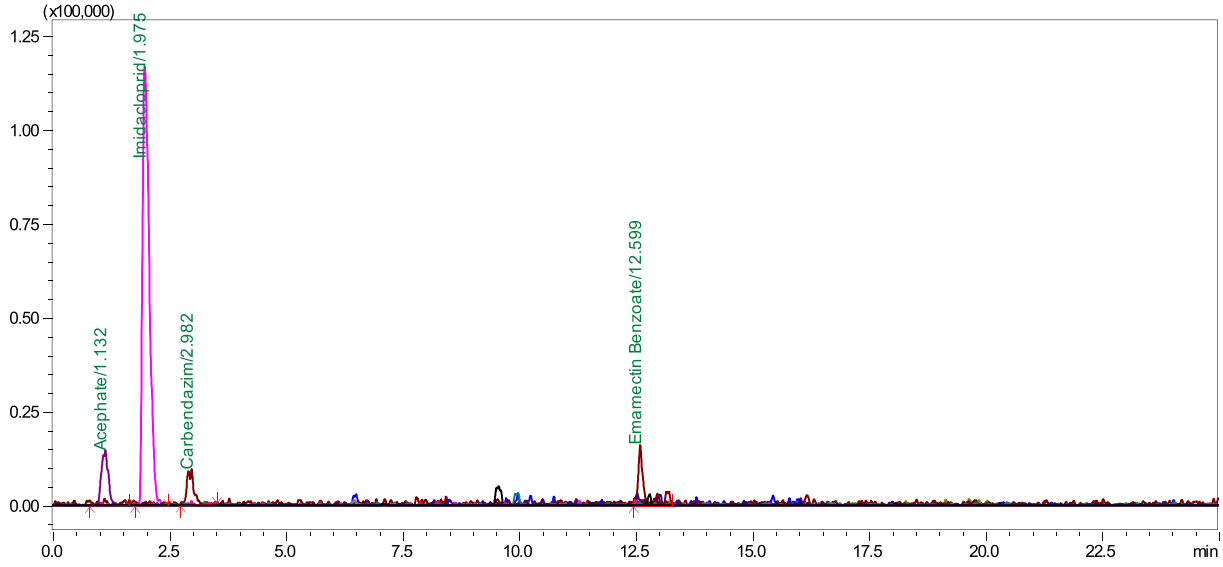

Fig. 5. LC-MS/MS chromatogram with detected insecticides in sample.

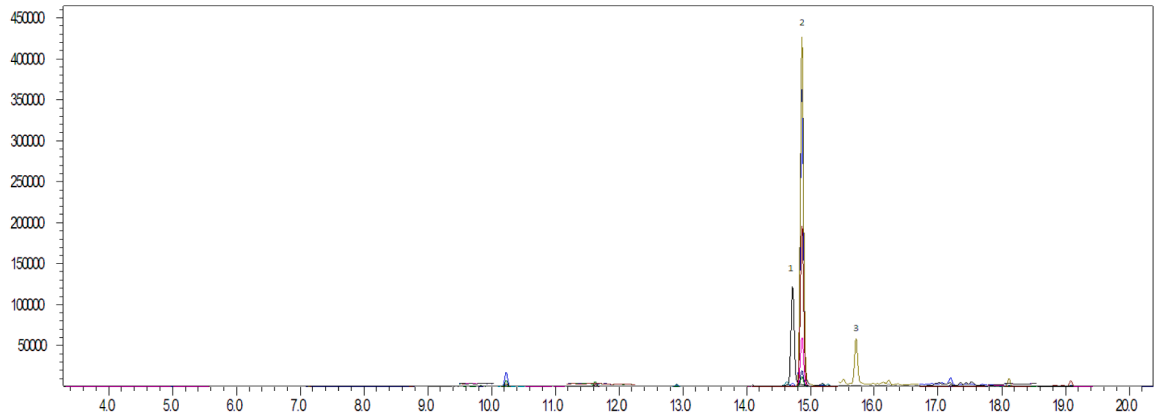

Fig. 6. Okra market sample chromatogram detected with 1. Bifenthrin, 2. Fenpropathrin, 3. Lambda-Cyhalothrin.

Jacob, & Anita, 2011). Previous multi-residue analysis in vegetable foods reflected the contamination of persistent synthetic pesticides of OC, OP, or SP molecules, and contamination is due to the frequent application of conventional group insecticides. Organophosphates and synthetic pesticides are major contaminants in commonly grown vegetables, *viz.*, okra and brinjal. It was observed that the fenitrothion (0.170 mg kg^{-1}) in okra, ethion (10.350 mg kg^{-1}), acephate (0.363 mg kg^{-1}), cypermethrin (0.002 mg kg^{-1}) and fenitrothion (0.475 mg kg^{-1}) in brinjal, respectively (Chowdhury et al., 2014).

After 2010, the pesticide contamination in market fruits and vegetables showed applied chemicals and conventional persistent pesticides. It was evident from a study of Sheikh et al. (2013) with different vegetable samples found contaminated with organophosphates (chlorpyrifos, profenofos), cyclodine (endosulfan), synthetic pyrethroids (bifenthrin, and cypermethrin), neonicotinoids (imidacloprid), avermectin (emamectin benzoate), insect growth regulator (diafenthiuron and lufenuron). However, all vegetables had more than one pesticide and violated the Japanese MRLs. Among many vegetables, okra is one of the significant vegetables marketed every day in India. It is grown with multiples pesticides applications, and monitoring the pesticides using the multi-residue method would facilitate the quantum of pesticide residues on marketable commodities offered for consumption. In similar studies, the insecticides *viz.,* monocrotophos, chlorpyriphos, and dimethoate residue analysis in farm gate okra samples showed 42 that, and 39% of the tested samples contained residues higher residues the MRL, respectively (Pal et al., 2016). The application of neonicotinoids in okra is a common practice in vegetable cultivation. Their high biological efficacy and frequent application could result in the occurrence of potential residues. Okra collected from three different vegetable from the Multan region of Pakistan showed the presence of imidacloprid and acetamiprid residues in 58 and 65 % samples, respectively, and with 10 and 15 % samples found residues above MRLs (Amjad et al., 2019).

Growing vegetables alongside a high pesticide intensive crop yield higher residues to the adjoining vegetable crops. It was observed that okra recorded an accumulation of significant residues when planted close to watermelon. The concentration ranged from 3.10 to 7.60, 2.80 to 2016.80, and 0.10 to 4.10 μg kg^{-1} for OC, OP, and SP groups, respectively, of which the residue levels for methamidophos, malathion, and dimethoate was 6.05, 23.30, and 50.60 μg kg^{-1}, respectively than tolerance limit advocated by WHO/FAO (Essumang et al., 2013). The *peri*-urban farming system of Pakistan recorded a high amount of pyrethroid residues. Synthetic pyrethroid residues have been extensively noticed in vegetables and fruits samples in China as well, and it is in the range of 1.60 to 1980.00 μgg^{-1} (Feng & Jin, 2007). Similarly, a deltamethrin level was 0.285 mg kg^{-1} in okra, 0.306 mg kg^{-1} in cauliflower, and 0.421 mg kg^{-1} in spinach (Randhawa et al., 2008). In the present study, the market and farm gate sample analysis detected with multiclass pesticides with moderate residues and multiresidue methods are essential with confirmation techniques such as LC-MS/MS and GC–MS/MS fulfills requirements of the global food standards.

5. Conclusions

In this research, a sensitive and reproducible method was developed and validated to simultaneously determine 73 pesticide residues in okra fruit using LC-MS/MS and GC–MS/MS. As per SANTE guidelines, the optimized methods provided good precision, precision-intra, and inter-day for all the selected pesticides. Further, the laboratory-validated data for measurement uncertainty estimation found a combined uncertainty (U_x) value in the range of 1.81 to 12.91 μg kg^{-1} evaluated at 50 μg kg^{-1}. The developed method was successfully adopted to screen the organophosphates, neonicotinoids, fungicides, insect growth regulators residues in real okra samples collected from field and market. Out of many pesticides detected in the field and market samples, only a pesticide showed risk to both consumer categories. This method can be employed for routine monitoring of okra fruits to meet the indigenous and export requirements. Further, the specific methods can be developed involving the pesticides of polar, non polar groups and pesticide which are currently used and frequently detected with special reference to the export importance. Methods are also been standardized to shorten the analysis time and covering more compounds with lowest level of control in GC–MS/MS and LC-MS/MS.

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CRediT authorship contribution statement

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Methodology, Validation, Visualization, Data curation, Writing – review & editing. **R. Udaykumar Nidoni:** Supervision, Resources, Methodology, Investigation, Validation, Project administration. **A. Prabhuraj:** Supervision, Resources, Funding acquisition, Visualization. **M. Bheemanna:** Supervision, Resources, Funding acquisition, Project administration, Software.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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