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Simultaneous determination of 79 pesticides in pigeonpea grains using GC–MS/MS and LC–MS/MS

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ABSTRACT

Pigeonpea grains are important sources of vegetarian proteins. It is the paramount importance to check the pesticide residues due to their frequent use during production. The LC–MS/MS and GC–MS/MS analytical method was developed and validated for the simultaneous determination of 79 pesticide residues in pigeonpea. The LOD and LOQ of the analytical method were in the range of 0.53 to 3.97 and 1.60 to 10.05 μg kg^{-1}, respectively, with a correlation coefficient of more than 0.997. Average recoveries were in the range of 80 to 118.8%, with the RSD of less than 15%. Measurement uncertainty (U_x) for pesticides was in the range of 3.42 to 12.76 μg kg^{-1} evaluated at 50 μg kg^{-1}. The method was applied to analyze the sample collected from the farmer's field. This method could be useful for routine analysis of selected pesticide residue for monitoring purposes.

1. Introduction

Pigeonpea is an important drought tolarent, perennial grain legume cultivated in the world's arid and semi-arid region for its protein-enriched seeds, fiber, and fuel (Shanower, Romeis, & Minja, 1999). It is being consumed as green seeds as a vegetable (immature pods or green pea) and reconstituted dried split seeds. It is an excellent source of dietary protein for vegetarian societies of South Asia (Ryan, Bidinger, Prahlad, & Pushpamma, 1984). It is the fifth prominent pulse crop globally and in India; it is being cultivated in an area of 5.33 mha with a production of 4.83 million tonnes (Sharma, Yelshetty, Kumari, Saini, & Chattopadhyay, 2014). Insect pests and disease are the major yield-limiting factors in pigeonpea production, realizing the expected yields (Shanower et al., 1999). The major insects' pests are pod borer (28%), spotted pod borer or Webber (20 to 60%), pod fly (13.5 to 33.2%), and pod bug complex (25 to 40%) causes a significant loss in grain yield (Sahoo & Senapati, 2000; Shanower et al., 1999; Sharma et al., 2014). Substantial yield losses in pigeonpea are being managed using chemical pesticides. Witnessing the visible knockdown effect of pesticides, more particularly by the insecticides on pod borer, pod fly, and pod bug complex, growers highly relying on chemical pesticides (Sharma et al., 2014; Tohnishi et al., 2005). The pesticides being used for containing various groups of pests may cause the occurrence of the potential residues above their MRLs and have toxicological significance in the environment. The MRLs set for pesticides, and plant protection chemicals used in pigeonpea are in the range of 0.01 to 1.50 mg kg^{-1} (FSSAI, 2018; EU Pesticides Database, 2016).

The monitoring of pesticide residues in pulses has revealed the detection of multiple pesticides (Ntow, Gijzen, Kelderman, & Drechsel, 2006) and the presence of organochlorine insecticides in grains, pulses, and nuts (Thompson, Hill, & Fishwick, 2006). Multi-residue analytical methods for pigeonpea grain, split dal, flour, and different other pulses for monitoring pesticides are essential using sample processing techniques like QuEChERS, which has previously developed and modified for analysis of pesticide residues in different food matrices (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; Lehotay, 2000; Lehotay, Kok, Hiemstra, & Van Bodegraven, 2005; Mastovska, Hajslova, & Lehotay, 2004; Mastovska, Lehotay, & Anastassiades, 2005). The analytical method for the estimation of pesticide residues in grain pulses and their products are scanty. A pesticide residue analysis method in the

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lentil was attended using LC–MS/MS (Iwakoshi et al., 2016). The method for estimating 71 plant protection chemicals in pulses was standardized (Banerjee, Gupta, Singh, & Narayanan, 2019). However, QuEChERS based multi-residue method for estimation of pesticide residues in cereals (He et al., 2015; Koesuwiwat, Lehotay, Mastovska, Dorweiler, & Leepipatpinoon, 2010); rice (da C. Cabrera, Caldas, Prestes, Primel, & Zanella, 2016); wheat grains, flour, and bran (Kolberg, Prestes, Adaime, & Zanella, 2011); fruits and vegetables (Eitzer, Hammack, & Filigenzi, 2014; Kmella, Abranko, Fodora, & Lehotay, 2010; Zhao, Wang, Luo, Li, & Pan, 2012) were developed previously. The method for analysis of flubendiamide and its metabolite, desiodo fluebendiamide analysis in pigeonpea grain, straw, and shell using HPLC (Battu, Singh, Kooner, & Singh, 2008); indoxacarb in pods and grains of pigeonpea using LC–MS/MS (Naik et al., 2020) and 71 plant protection chemicals in pulses (Banerjee et al., 2019) are the latest report for the residue analytical methods in pulses. Available literature with this commodity is very less. There is a great need for monitoring of the pesticide residues in the pigeonpea grains. It is essential to standardize the multi-residue analytical method in pigeonpea grains for use in the routine monitoring of pesticides.

For any validated method, which is being used for the analysis of samples in the regulatory laboratory, the estimation of measurement uncertainty is a pre-requisite. The quality attribute of a measurement result, and uncertainty value is such that the measurement results are fit for the intended purpose. Therefore correct estimation of measurement result and its associated uncertainty is essential to confirm the optimized method for sample preparation and analysis is efficient for simultaneous determination of pesticide contaminants in food grains and other complex matrices (Ellison & Williams, 2012; NABL 141, 2016; Banerjee et al., 2007; Kanrar, Mandal, & Bhattacharyya, 2010). This ensures that risks associated with compliance decisions are within acceptable limits. Proficiency testing in trace level contaminant analysis is important to understand the statistical limitations through external means of quality assessment when gauging the laboratory's competence and methods for the analysis of samples and reporting the analytical results (NABL 162, 2008). Accurate detection and quantification of pesticides in the check samples using the developed method ensure quality assurance in which the false positive and false negative reports are avoided (Sharma, 2013). With this background, an analytical method was developed and validated in LC–MS/MS, and GC–MSMS to determine 79 chemical pesticides involving modified QuEChERS extraction techniques and estimated the measurement uncertainty. Overall, this method could be useful for large-scale monitoring of pesticide which is currently being used in pulses production and including some of the high persistence pesticides of organochlorine and organophosphate group, despite their usages have been stopped or restricted in agriculture.

2. Materials and method

2.1. Blank matrix and pesticide selection

The pigeonpea grain sample was collected from the organic farmer field and screened for pesticide contamination. The grain sample was prepared using a high volume blender (Robo Coup) and stored at 8 °C (walk-in cold chamber) for further analysis. Pesticides selected in this study represent organochlorine, organophosphates, carbamates, synthetic pyrethroids, neonicotinoids, herbicides, fungicides, bio-rational, and new chemistry groups molecules.

2.2. Chemical and reagents

Certified reference materials of selected pesticides in this study (≥98.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). LC–MS grade of acetonitrile, ethyl acetate, methanol (≥99.9%), and sodium chloride (≥99%) were procured from Merck, Mumbai (India). Anhydrous magnesium sulfate, sodium acetate, sodium sulfate (>99%), and diethylene glycol were obtained from Himedia, Bangalore, India. Ammonium formate and formic acid (90%) were purchased from Empart, Hyderabad (India). Primary, secondary amine (PSA, 40 μm) was obtained from Agilent Technologies India Pvt. Ltd., Bangalore, India. Milli-Q water system was used to draw the HPLC grade water.

2.3. Pesticide standard solutions

The standard stock (1000 μg mL^{-1}) solution was prepared by dissolving approximately 10.00 ± 0.1 mg of individual pesticide CRM in a 10 mL volumetric flask (Class A and calibrated) and dissolved with methanol and ethyl acetate for LC and GC analysis, respectively. An intermediate working standard solution of 100 μg mL^{-1} was prepared by drawing 1 mL of stock solution in 10 mL volumetric flask and volume made with solvents. A working standard mixture of 10 μg mL^{-1} was prepared using an intermediate stock solution. Further calibration standard solutions ranging from 10 to 1000 μg L^{-1} were prepared. The matrix match standards with similar concentrations were prepared by using the blank sample matrix extract.

2.4. Sample preparation, extraction, and clean-up.

The pigeonpea matrix was extracted and cleaned-up following the modified QuEChERS procedure based on the methods given by Anastassiades et al. (2003), Lehotay et al. (2005) and Lehotay (2007). A representative 5 g of the sample was weighed and transferred into a 50 mL centrifuge tube. To this, 10 mL of distilled water was added and allowed to stand for 30 min. Further, 10 mL of 1% ethyl acetate in acetonitrile, 6 g of anhydrous magnesium sulfate, and 1.5 g sodium acetate were added. The sample mixture was then homogenized at 10000–13000 rpm for 3 min and centrifuged at 2800×*g* for 5 min. at 10 °C. After centrifugation, 7 mL supernatant was transferred into a 15 mL centrifuge tube containing 0.35 g primary, secondary amine, and 1.05 g magnesium sulfate, vortexed the mixture for one minute followed by centrifugation at 16128×*g* for 5 min. Then 3 mL extractant was transferred in two test tubes containing 300 μL of 10% diethylene glycol separately for LC and GC analysis and evaporated the content near to dryness using nitrogen flash evaporator at 35 °C. Later reconstituted the residue with 1.5 mL methanol and ethyl acetate for LC–MS/MS and GC–MS/MS analysis. Sonicated the mixture using an ultrasonicator to dissolve residues completely and filter the content using a 0.22 μ PTFE nylon filter in the vials.

2.5. LC–MS/MS parameters

LC–MS 8040 model from the Shimadzu® was used in this study. LC–MS/MS system equipped with UHPLC (Shimadzu 1200) and triple quadrupole MS/MS detector employed for analysis, separation, and confirmation of pesticides. LabSolution® software (1.5 version) was employed for data acquisition, processing, and overall controlling of the instrument. Shimpack XR, octadecylsilyl (ODS III) C18 column (2 mm i. d × 150 mm, 2.2 μm particle size) was used for chromatographic separation. Analytical conditions for LC as follow; 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 with mobile phase flow rate of 0.4 mL min^{-1} and injection volume 2 μL. The column temperature was maintained at 40 °C. The mobile phase gradients program for the binary pump was 60% A and 40% B initially for 15 min. followed by 100% B for 5 min. and then 60% A and 40% B for 5 min. The cycle time and total time program were 0.234 *sec* and 25 min., respectively. The mass spectrometry parameter as follows; the sample was ionized using electro spray ionization (ESI+) mode, 0.1 μÅ of interface current, 400 °C of heat block temperature, 250 °C of DL temperature were maintained. Nitrogen (N_2, 99.99%) as nebulizer gas of

2.9 L min^{-1} and drying gas of 15 L min^{-1}, CID gas as argon (Ar, 99.999%) of 230 kpa, scan speed was 6000 u/*sec* was employed. Dwell time was 1 msec. Pesticide (analyte) fragmentation, voltage, and the collision energy were optimized for obtaining the highest sensitivity in the developed method.

2.6. GC–MS/MS parameters

GC–MS TQ8030 model (Shimadzu®) with MS/MS triple quadrupole system coupled with an electronic flow controller (EFC) was used in this study. GC system (GC 2100) with an AOC-20i injector and AOC-20s autosampler were employed. Instrument control, data acquisition, and processing were performed with LabSolution® software. Chromatographic separation of pesticides was made using a capillary fused silica HP 5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The splitless injection mode with the volume of 2 μL, surge pressure of 250 kpa, and injector temperature of 280 °C was maintained. The column oven temperature program as follows, initially, set at 60 °C held for 2 min, 25 °C min^{-1} rate to 150 °C, then 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. Helium (99.999% purity) was used as carrier gas with a constant flow rate of 1.5 mL min^{-1}. MS parameters such as transfer line temperature (280 °C) and ion source temperature (250 °C) were maintained. Samples were ionized in MS/MS 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 mL min^{-1} were used as collision and quench gases. Mass range was between 50 and 550 *m/z* with 1666 u/*sec* scan speed. The total time program was 40 min.

2.7. Method validation

The method was validated following the European commission guideline SANTAE/11813/ 2017 (European Commission, 2017). Linearity study was conducted by preparing the pesticide standard in the range of 10 to 1000 μg L^{-1} (10, 50, 100, 250, 500, and 1000 μg L^{-1}) in solvent and pigeonpea blank matrix and injected into LC–MS/MS and GC–MS/MS. The linearity graph was drawn, and correlation coefficient for each pesticide was recorded. Matrix effect was calculated using angular co-efficient from the linearity study with pigeonpea matrix match standard and pure solvent. A higher and lower slope in the matrix calibration indicated the matrix-induced signal enhancement and suppression, respectively, and it was calculated using the following mathematical formulae.

Matrix effect (%) = (bm − bs)/bs × 100, Where bm and bs are the angular coefficient of the curve in the matrix and the solvent, respectively (da Silva, Habermann, Marchi, & Zocolo, 2012).

The LOD and LOQ were the lowest concentration of pesticide mixture at which the analyte could reliably detect and quantified through GC–MS/MS and LC–MS/MS, respectively. These were identified by spiking the lowest concentration of pesticide mixture to the pigeonpea blank matrix (n = 6). 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 (Magnusson & Örnemark, 2014; Wenzl, Haedrich, Schaechtele, Robouch, & Stroka, 2016). The mathematical formulae employed as follow

$$X_{LOD} = 5.2 \times S_y, \text{ net/b}$$

$$X_{LOQ} = 3.3 \times X_{LOD}$$

where, X_{LOD}: Limit of detection

$S_{y,net}$: Standard deviation of the precision data at the lowest spike level
b: Slope of the calibration curve
X_{LOQ}: Limit of quantification

Calculated LOD and LOQ were given in Table 2. However, most of the LOQ were less than 10 μg kg^{-1} except few pesticides (some are equal to 10 μg kg^{-1} or slightly above) as per the above equation. The LOQs must comply with the tolerance limits fixed by different countries to assess the quality of feed and food. For pigeonpea, the MRLs for all the tested pesticides were found more than 10 μg kg^{-1} and up to 1000 μg kg^{-1}. In this study, a practical approach to achieve acceptable precision at the lowest spiking level, which is less than MRLs of all the tested analytes, the pigeonpea matrix was spiked at 10 μg kg^{-1}, and recovery results were calculated and defined as the lowest validated spike level meeting the requirement of recovery and relative standard deviation (RSD) for different fortification levels.

The recovery experiments were carried out on control (blank) samples of pigeon pea grain by spiking the mixed standard solution of selected pesticides at various concentrations (10, 50, and 100 μg kg^{-1}), and each level replicated six times. The recoveries were calculated using matrix match standards. Precision in terms of repeatability-intraday (Repeatability-RSDr) and interday (Reproducibility-RSDwr) was attended at the concentration of 50 μg kg^{-1}. Intraday precision was carried out with six replication of spiked samples and extracted, injected into instruments. Interday precision was carried out on two different days at six replications each and calculated the percent recovery and RSD with results obtained between two different dates. Whereas, ruggedness for the method was attended by fortifying the 6 individual samples at 50 μg kg^{-1} and extracted and analyzing the samples by two different analysts. The recovery and RSD (%) were compared for both analysts.

2.8. Evaluation of measurement of uncertainty

The expanded uncertainty (U_{exp}) at 95% confidence limit was calculated for fortification level of 50 μg kg^{-1} as per the statistical method mentioned in EURACHEM/CITAC Guide (Ellison & Williams, 2012; NABL 141, 2016; Banerjee et al., 2007; Kanrar et al., 2010). Different sources of uncertainty were taken into accounts, such as type 'A' uncertainty (U), combined uncertainty (U_c), and expanded uncertainty (U_{exp}), and their stepwise estimation procedure has been explained in Table S1. The equation for calculation of type A and B uncertainty is as follow,

Uncertainty due to repeatability (Type A Uncertainty),

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

Combined Uncertainty (Type 'B' Uncertainty),

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

Coverage factor (*k*) at 95% Confidence level

$$V_{effective} = \frac{(U_C)^4}{\frac{(U_1)^4}{df.} + \frac{(U_2)^4}{df.} + \cdots + \frac{(Un)^4}{df.}}$$

Table 1
Retention time, mass spectrometric parameters for multiclass pesticides analyzed in pigeonpea.

Peak No.	Pesticide	Retention time (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								
1	Dichlorvas	6.41	185.0 > 93.0	14	185.0 > 109.0	14	185.0 > 63.0	22
2	Phenol, 4-bromo-2-chloromo	6.56	208.0 > 63.10	27	208.0 > 99.10	21	208.0 > 144.00	15
3	Trifluralin	12.34	110.1 > 64.0 18	18	152.1 > 110.1	8	110.1 > 92.0	12
4	Alpha-BHC	12.81	306.1 > 264.1	8	306.1 > 206.1	14	264.1 > 206.1	8
5	Beta-BHC	14.04	218.9 > 182.9	8	180.9 > 144.9	16	180.9 > 74.0	30
6	Diazinon	15.17	304.1 > 179.1	10	304.1 > 162.1	8	304.1 > 137.1	26
7	Fluchloralin	15.38	306.0 > 264.10	6	306.0 > 160.20	27	306.0 > 206.20	18
8	Delta-BHC	15.36	218.9 > 182.9	10	218.9 > 144.9	20	180.9 > 144.9	16
9	Tri-allate	15.67	268.1 > 226.0	14	268.1 > 184.0	20	270.1 > 228.0	14
10	Iprobenfos	16.06	204.0 > 91.0	8	204.0 > 171.0	6	123.0 > 45.0	16
11	Propanil	16.87	160.9 > 99.0	24	217.0 > 161.0	10	217.0 > 57.0	20
12	Chlorpyrifos-methyl	17.31	285.9 > 93.0	22	287.9 > 272.9	20	287.9 > 93.0	22
13	Parathion-methyl	17.30	125.0 > 47.0	12	263.0 > 109.0	14	125.0 > 62.0	6
14	Alachlor	17.73	188.1 > 160.1	10	188.1 > 132.1	18	160.1 > 132.1	10
15	Heptachlor	17.50	271.8 > 236.9	20	271.8 > 117.0	32	271.8 > 201.9	38
16	Fenitrothion	18.77	277.0 > 260.0	6	277.0 > 109.1	14	260.0 > 125.1	22
17	Aldrin	19.20	292.9 > 219.9	26	292.9 > 257.9	16	292.9 > 186.0	40
18	Chlorpyrifos	19.91	313.9 > 257.9	14	313.9 > 285.9	8	313.9 > 193.9	28
19	Ethyl Parathion	19.95	291.1 > 109.0	14	291.1 > 137.0	6	291.1 > 81.0	24
20	Chlorfenvinphos	22.21	267.0 > 159.0	18	323.0 > 267.0	16	323.0 > 295.0	6
21	Phenthoate	22.34	119.1 > 82.1	28	119.1 > 84.1	28	149.1 > 105.1	4
22	Allethrin	22.39	273.9 > 125.0	20	246.0 > 121.0	6	246.0 > 63.0	28
23	alpha-Endosulfan	23.21	338.9 > 160.0	18	338.9 > 195.9	20	194.9 > 125.0	24
24	Butachlor	23.76	188.1 > 160.1	12	188.1 > 132.1	18	176.1 > 134.1	12
25	p,p'-DDE	24.53	246.0 > 176.0	30	246.0 > 211.0	22	317.9 > 248.0	24
26	Endrin	25.22	262.9 > 193.0	28	262.9 > 228.0	22	244.9 > 210.0	8
27	Beta-Endosulfan	25.62	338.9 > 160.0	18	338.9 > 266.9	8	338.9 > 195.9	20
28	p,p'-DDT	26.12	235.0 > 165.0	24	235.0 > 199.0	16	237.0 > 199.0	16
29	Endosulfan sulfate	27.13	386.8 > 288.8	10	386.8 > 252.9	16	386.8 > 240.9	22
30	o,p'-DDT	27.34	235.0 > 165.0	24	235.0 > 199.0	16	237.0 > 199.0	16
31	Bifenthrin	29.08	181.1 > 166.1	12	181.1 > 153.1	8	181.1 > 179.1	12
32	Fenpropathrin	29.23	265.1 > 210.1	12	265.1 > 89.0	28	181.1 > 127.1	28
33	Lambda-Cyhalothrin	30.55	181.1 > 152.1	24	163.1 > 127.0	14	–	–
34	Permethrin I	31.49	163.1 > 127.1	8	183.1 > 165.1	14	183.1 > 153.1	14
35	Permethrin II	31.67	163.1 > 127.1	8	183.1 > 165.1	14	183.1 > 153.1	14
36	Cypermethrin	30.55	163.1 > 91.0	14	181.1 > 152.1	22	181.1 > 127.1	22
37	Cyfluthrin	32.56	226.1 > 206.1	14	226.1 > 199.1	6	163.1 > 91.0	14
38	Etofenprox	33.16	163.1 > 135.1	10	163.1 > 107.1	18	163.1 > 95.0	18
39	Fenvalerate	34.20	419.1 > 225.1	6	419.1 > 167.1	12	419.1 > 125.1	26
40	Deltamethrin	35.64	252.9 > 93.0	20	252.9 > 171.9	8	252.9 > 77.0	26
LC–MS/MS								
1	Acephate	1.06	183.9 > 142.95	11	183.9 > 49	23	183.9 > 95.05	26
2	Omethoate	1.09	214 > 125	24	214 > 182.95	12	214 > 109.05	30
3	Pymetrozine	1.32	217.9 > 105.1	22	217.9 > 78	44	217.9 > 51.15	54
4	Imidacloprid	2.03	256.05 > 209.1	17	256.05 > 221.15	9	256.05 > 175.05	19
5	Dimethoate	2.67	229.95 > 199	10	229.95 > 125	22	229.95 > 171	17
6	Carbendazim	3.05	191.95 > 160	18	191.95 > 132	32	191.95 > 105.05	40
7	Thiacloprid	3.22	253.2 > 126.05	23	253.2 > 99.1	48	253.2 > 90.05	40
8	Phosphomidon	4.65	299.9 > 174.1	14	299.9 > 127.05	28	299.9 > 132.1	24
9	Bendiocarb	5.42	224 > 167.1	11	224 > 109	19	224 > 81.1	35
10	Metribuzin	5.43	215 > 187.1	20	215 > 49	29	215 > 58.05	27
11	Carbofuron	5.47	221.8 > 165.1	12	221.8 > 123.1	23	221.8 > 55.05	29
12	Carbaryl	6.12	202 > 145.05	12	202 > 127	28	202 > 117.1	24
13	Isoproturon	7.48	206.9 > 72.1	23	206.9 > 46.15	18	206.9 > 165.1	15
14	Metalaxyl	7.52	279.8 > 220.2	15	279.8 > 192.15	18	279.8 > 248.15	11
15	Chlorantraniliprole	8.31	483.75 > 452.95	17	483.75 > 285.9	16	483.75 > 177.05	50
16	Coumatetryl	9.11	293.1 > 175.1	24	293.1 > 91.15	36	293.1 > 107.05	35
17	Paclobutrazole	9.47	294.25 > 70.15	23	294.25 > 125.1	37	294.25 > 43.15	50
18	Methoxyfenozide	9.63	368.95 > 149.05	18	368.95 > 313.15	9	368.95 > 133.05	24
19	Triademefon	9.72	293.9 > 69.1	23	293.9 > 197.1	17	293.9 > 225.1	14
20	Triazophos	9.98	313.65 > 162.1	19	313.65 > 119.1	35	313.65 > 97.05	37
21	Triademenol	10.04	296.25 > 70.05	12	296.25 > 43.15	47	296.25 > 99.1	18
22	Tetraconazole	10.4	371.8 > 159.05	34	371.8 > 70.15	25	371.8 > 150.1	35
23	Metalachlor	10.64	283.95 > 252.15	15	283.95 > 176.2	27	283.95 > 134.1	34
24	Quinalphos	11.2	299.2 > 163.15	22	299.2 > 147.1	22	299.2 > 119	44
25	Penconazole	11.37	284.25 > 70.15	18	284.25 > 159.05	31	284.25 > 267.2	8
26	Benalaxyl	11.55	325.8 > 148.15	23	325.8 > 294.15	12	325.8 > 208.1	16
27	Hexaconazole	11.86	314.1 > 70.15	22	314.1 > 297.2	10	314.1 > 45.1	39
28	Bitertenol	12.03	338.2 > 269.2	10	338.2 > 99.1	17	338.2 > 70.15	11

(*continued on next page*)

Table 1 (*continued*)

Peak No.	Pesticide	Retention time (min)	Quantification		Confirmation			
			MRM transition (*m/z*)	Collision Energy (eV)	MRM transition (*m/z*)	Collision Energy (eV)	MRM transition (*m/z*)	Collision Energy (eV)
29	Phosalone	12.05	367.7 > 182.05	16	367.7 > 111.05	43	367.7 > 138.1	32
30	Spinosad	12.17	732.2 > 142.15	35	732.2 > 98.25	54	732.2 > 99.15	52
31	Thiobencarb	12.25	258.05 > 125.05	20	258.05 > 100.15	13	258.05 > 89.15	49
32	Difenconazole	12.46	406.05 > 251	28	406.05 > 111.05	55	406.05 > 188.05	47
33	Pretilachlor	12.81	311.9 > 252.15	16	311.9 > 176.2	29	311.9 > 147.1	40
34	Profenofos	13.13	374.95 > 304.9	21	374.95 > 346.95	14	374.95 > 128.15	47
35	Emamectin benzoate	13.31	886.3 > 158.15	42	886.3 > 126.15	48	886.3 > 82.2	50
36	Buprofezin	13.51	306.15 > 200.9	11	306.15 > 57.15	30	306.15 > 116.1	16
37	Hexythiazox	14.00	352.9 > 228	15	352.9 > 168.15	28	352.9 > 116.15	43
38	Pendimethalin	14.18	281.8 > 212.05	11	281.8 > 199.95	8	281.8 > 193.95	20
39	Fenpyroximate	14.62	422 > 366.2	16	422 > 138.2	35	422 > 215.15	27

MRM-Multiple Reaction Monitoring.

where,

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

Expanded Uncertainty (at 95% confidence; $k = 2$), $U_{exp} = U_c \times k$
where,

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

2.9. Proficiency tests

A pulse sample (chickpea) was received under the proficiency testing program conducted by the National Institute of Plant Health Management, Hyderabad, India. The sample was extracted and clean-up as per the procedure described in the previous section. The 'Z' score for the reported pesticides was calculated using the following mathematical formulae.

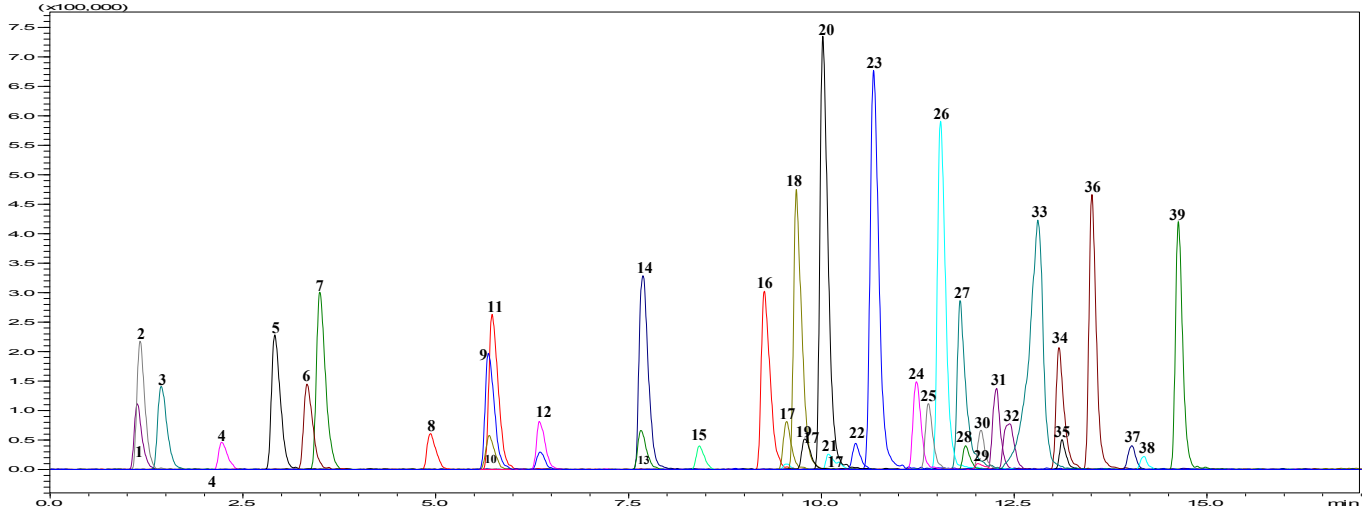

Fig. 1. LC–MS/MS chromatogram for 39 pesticide mixture at concentration of 0.1 μg mL^{-1}.

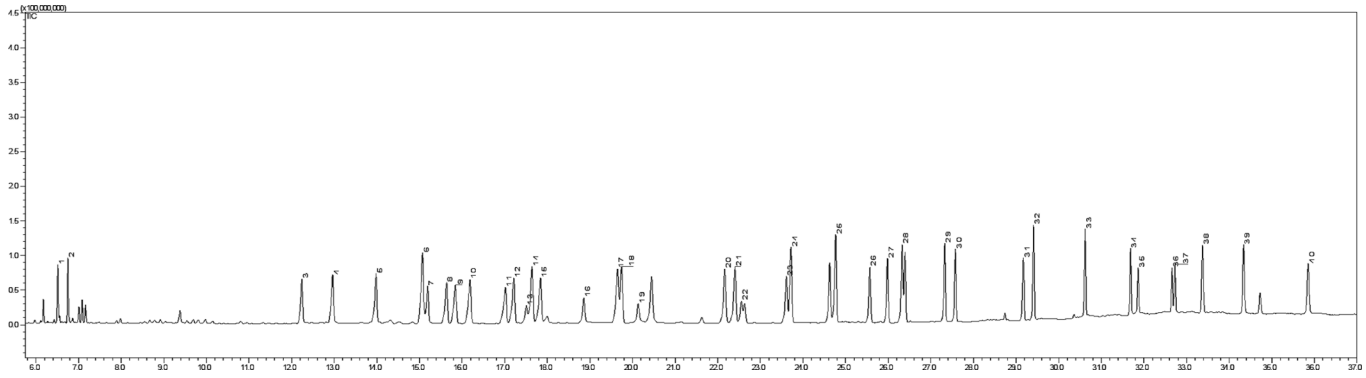

Fig. 2. GC–MS/MS chromatogram for 40 pesticides mixture at concentration of 0.1 μg mL^{-1}.

Table 2
Regression equation, LOD, LOQ and recovery of multiclass pesticides and their metabolites in pigeonpea grains.

Pesticides	Regression equation	R^2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)		
					Fortification levels (μg kg^{-1})		
					10	50	100
Dichlorvos	y = 1526.77X + 3525.043	0.998	1.15	3.44	85.4	89.5	88.3
Phenol, 4-bromo-2-chloro-	y = 2213.594X + 6749.038	0.999	2.04	6.13	104.2	84.3	92.6
Trifluralin	y = 2614.717X − 310.6054	0.998	3.35	10.05	109.8	89.8	87.9
Alpha-BHC	y = 1935.408X − 276.7023	0.998	1.92	5.75	106.9	95.0	89.7
Beta-BHC	y = 1678.096X − 1654.748	0.999	1.19	3.56	103.3	87.2	97.0
Diazinon	y = 1146.529X − 2449.562	0.997	3.35	10.03	98.2	89.9	96.1
Fluchloralin	y = 1217.287X − 395.9018	0.999	3.33	10.01	101.9	88.9	99.8
Delta-BHC	y = 625.2097X − 2530.381	0.998	3.35	10.04	97.2	103.4	104.2
Tri-allate	y = 1132.621X − 275.9769	0.999	3.54	10.05	103.4	109.3	100.5
Iprobenfos	y = 7372.69X + 1295.327	0.999	1.78	5.34	107.2	94.7	96.8
Propanil	y = 3012.521X + 1333.48	0.997	1.88	5.65	99.2	89.4	88.0
Chlorpyrifos-methyl	y = 1580.833X − 57.43438	0.999	1.45	4.35	92.3	96.0	99.8
Parathion-methyl	y = 3645.641X + 1308.131	0.999	1.75	5.25	95.4	85.3	93.3
Alachlor	y = 1575.606X − 3341.53	0.998	2.64	7.93	101.2	106.9	95.3
Heptachlor	y = 2398.995X − 2074.688	0.998	1.90	5.69	91.6	88.4	93.0
Fenitrothion	y = 1416.766X − 713.8973	0.998	2.96	8.89	105.8	91.2	87.7
Aldrin	y = 704.9229X − 47.22827	0.999	2.29	6.87	99.2	88.7	89.6
Chlorpyrifos	y = 1799.683X + 1304.947	0.999	3.33	10.00	98.2	88.6	97.7
Ethyl Parathion	y = 1038.502X − 2460.769	0.998	3.08	9.25	102.5	106.9	87.0
Chlorfenvinphos	y = 2205.937X − 862.9641	0.999	1.79	5.36	97.9	86.8	94.5
Phenthoate	y = 2178.061X − 737.0937	0.998	1.91	5.74	99.3	93.8	98.9
Allethrin	y = 1079.048X + 9540.553	0.997	2.37	7.11	102.6	86.5	91.3
Alpha-Endosulfan	y = 102.547X − 549.2491	0.998	2.04	6.13	98.7	93.3	88.7
Butachlor	y = 1564.909X + 3626.707	0.998	1.81	5.44	92.6	104.2	98.9
p,p'-DDE	y = 6016.196X + 3577.863	0.997	1.37	4.10	100.1	86.7	97.7
Endrin	y = 513.2413X + 1534.321	0.999	2.35	7.06	101.8	86.1	92.6
Beta-Endosulfan	y = 82.98231X − 364.034	0.998	3.97	9.91	94.2	93.9	97.6
p,p'-DDT	y = 7932.269X − 14919.3	0.998	1.05	3.15	103.1	95.5	98.2
Endosulfan sulfate	y = 46.91415X − 237.7651	0.997	3.63	9.89	105.4	100.2	87.8
o,p'-DDT	y = 2342.272X − 5884.521	0.998	1.32	3.96	85.9	88.3	87.6
Bifenthrin	y = 22720.25X + 25964.12	0.999	0.92	2.76	98.6	84.9	88.6
Fenpropathrin	y = 1188.696X + 481.6334	0.999	0.83	2.50	93.4	87.9	102.7
L-Cyhalothrin	y = 2579.178X + 2346.444	0.997	3.19	9.57	87.0	95.1	86.3
Permethrin-1	y = 2313.364X + 20203.78	0.999	3.08	9.23	100.2	90.0	98.8
Permethrin-II	y = 2315.035X + 20196.75	0.999	2.22	6.65	104.7	90.1	99.3
Cypermethrin	y = 771.9878X − 1440.54	0.997	0.80	2.40	105.4	86.7	85.0
Cyfluthrin	y = 1019.521X + 1909.735	0.998	3.95	9.86	90.0	93.5	88.6
Etofenprox	y = 21018.85X + 27438.5	0.998	0.71	2.14	97.2	102.4	96.4
Fenvalerate	y = 139.617x − 743.1964	0.997	2.04	6.11	94.5	84.5	88.0
Deltamethrin	y = 298.0482x + 2451.6	0.998	3.32	9.96	98.5	94.4	87.1
Acephate	y = 126853x + 309341	0.999	2.13	6.40	101.2	87.3	89.1
Benalaxyl	y = 115346x − 122,407	0.998	3.34	10.02	107.0	87.8	107.1
Bendiocarb	y = 176594x − 180,657	0.998	1.69	5.06	111.1	108.3	110.1
Bitertenol	Y = 18125.9X + 59806.2	0.999	2.38	7.13	111.6	90.2	85.5
Buprofezin	y = 64184x + 75753	0.999	2.88	8.63	117.4	112.9	98.4
Carbaryl	y = 53814x − 237,801	0.999	2.58	7.73	101.0	105.9	96.5
Carbendazim	y = 18362x + 32361	0.999	1.88	5.63	88.7	88.9	97.8
Carbofuron	y = 25889x − 196,755	0.999	3.91	9.73	114.5	117.5	93.6
Chlorantraniliprole	y = 49403x + 119318	0.999	2.07	6.20	114.9	102.7	101.1
Coumatetryl	y = 32191x − 248,335	0.999	1.21	3.63	98.6	93.5	98.6
Difenconazole	y = 17325x − 138,342	0.998	1.37	4.11	109.2	100.1	94.2
Dimethoate	y = 67389x − 690,837	0.999	2.48	7.45	115.8	109.3	91.3
Emamectin benzoate	y = 28935x + 175967	0.997	2.56	7.67	105.7	92.6	89.3
Fenpyroximate	Y = 148517x − 410536	0.999	2.71	8.13	117.0	110.3	107.0
Hexaconazole	y = 176163x − 1E + 06	0.998	1.95	5.85	99.1	85.9	93.6
Hexythiazox	y = 46042x + 30209	0.998	3.33	10.01	87.1	113.0	89.1
Imidacloprid	y = 56061x − 396,401	0.998	2.40	7.20	109.4	108.0	105.6
Isoproturon	y = 54423x + 69706	0.999	1.80	5.41	116.1	98.3	89.9
Metalachlor	y = 17815x − 160,829	0.998	1.34	4.02	115.8	101.3	88.4
Metalaxyl	y = 57652x + 641381	0.999	3.29	9.97	113.6	104.9	109.8
Methoxyfenozide	y = 47660x − 176,447	0.998	2.99	8.97	111.7	93.1	97.6
Metribuzin	y = 41716x + 9493.9	0.998	2.34	7.01	113.2	117.3	107.4
Omethoate	y = 63000x + 250316	0.999	2.48	7.43	104.2	79.1	107.7
Paclobutrazole	Y = 36414.5x − 49759.2	0.998	0.79	2.37	113.5	112.5	89.5
Penconazole	y = 17208x + 169542	0.999	2.36	7.09	114.6	101.9	91.1
Pendimethalin	y = 43309x − 366,972	0.998	2.00	5.99	89.0	104.3	95.1
Phosalone	y = 29132x − 231,318	0.999	3.32	9.97	113.5	116.3	89.6
Phosphomidon	Y = 22216.9x − 11599.6	0.999	2.80	8.41	110.4	113.7	102.0
Pretilachlor	y = 42466x + 305642	0.998	2.63	7.88	114.2	85.3	100.4
Profenofos	y = 28581x − 166,894	0.999	2.10	6.31	117.1	109.7	112.7
Pymetrozine	y = 21410x − 79,884	0.999	0.53	1.60	88.5	98.4	88.1

(*continued on next page*)

Table 2 (*continued*)

Pesticides	Regression equation	R^2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)		
					Fortification levels (μg kg^{-1})		
					10	50	100
Quinalphos	y = 13633x + 89667	0.998	2.01	6.04	113.4	118.8	98.2
Spinosad	Y = 41479.0x − 58952.1	0.999	1.79	5.37	114.8	90.2	93.8
Tetraconazole	y = 101901x − 293,965	0.999	3.31	9.93	116.1	112.6	116.2
Thiacloprid	y = 49464x + 484210	0.998	1.87	5.60	109.6	95.6	93.8
Thiobencarb	Y = 12430.0X − 13590.6	0.999	2.33	6.98	114.5	95.4	88.6
Triademefon	y = 40681x − 103,843	0.999	1.50	4.51	108.1	104.8	95.5
Triademenol	y = 30326x + 9962.7	0.999	2.70	8.10	117.3	101.7	99.4
Triazophos	y = 30911.3X + 105032	0.999	3.34	10.02	101.2	98.0	102.2

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

where X_i is the participants value; X_{pt} is the assigned value; σ_{pt} is the standard deviation for proficiency assessment. The modified Z score (Z' score) was calculated using formulae when there is a concern about the assigned value, i.e. $u(X_{pt})$, $> 0.3\ \sigma_{pt}$,

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

where, $u(X_{pt})$ = standard uncertainty of assigned value (NABL 162, 2008). The standard deviation for proficiency assessment (μg g^{-1}) and uncertainty of assigned value (μg g^{-1}) for all test parameter was added while calculating the Z score.

2.10. Monitoring of residue in real samples

A total of 50 pigeonpea grain samples were collected from the farmers field at the time of harvest. The samples were prepared, extracted, and clean-up, as described in the previous section. All samples were kept in suitable containers and stored at less than 2–8 °C for further analysis. The samples were tested for the pesticide residues using a validated method and quantified the residues. In order to observe the intra-matrix variation within the samples, the relative matrix effect was determined by comparing the responses obtained in the blank extract from the sample with matrix-matched standards and the per cent relative matrix effect was calculated (Steiner, Krska, Malachova, Taschl, & Sulyok, 2020).

3. Results and discussion

3.1. Optimization LC–MS/MS parameters

The thermally unstable and amenable for analysis with LC–MS/MS were optimized in electro spray ionization (ESI+) with the positive mode. MRM experiments were conducted with a heat block temperature of 400 °C. Firstly, the MS/MS conditions were optimized by direct injection of 0.1 μg mL^{-1} working solutions both in positive and negative ESI mode, respectively, for all the pesticides and optimization of the different precursor ion $[M+H]^+$, product ions, and collision energies. The highest sensitivity and optimal selectivity (with set LC–MS/MS parameter) for each pesticide were selected and established the multiple reaction monitoring (MRM) mode. The most intense transition (*m/z*) was used for quantitation, while the daughter ions (*m/z*) were employed for confirmation. The optimized parameters are presented in Table 1 and Fig. 1.

3.2. Optimization GC–MS/MS parameters

The chromatographic conditions described for the GC–MS/MS allowed an efficient separation for all the thermally stable and volatile pesticides. For method standardization, more than two transitions were selected for each pesticide to determine the suitable transitions for qualification and quantification. For most of the pesticides, the predominant intense product ion (*m/z*) was selected for quantification, and the other two ions (*m/z*) with less interference were selected as MRMs for confirmation having optimal specificity and sensitivity. After the selection of the precursor and product ions, the different collision energy was identified (Table 1). In this work, two product ions resulting from fragmentation of one precursor ion were monitored. With the set operating chromatographic conditions, the selected pesticides were separated and eluted at different retention times (Fig. 2), and total elution occurred in less than 36 min in the total run time of 40 min.

3.3. Method validation

The satisfactory regression equations and coefficients of determination (R^2) were obtained for all the tested pesticides (Table 2). The linear concentration of pesticide mixture such as 10, 50, 100, 250, 500 and 1000 μg L^{-1}, produce a linear relationship between detector response (y) and analyte concentration (x). The selected chromatographic conditions for pesticides have recorded the coefficient of determination (R^2) in the range of 0.997 to 0.999. The LOD for the pesticides were in the range of 0.53 to 3.97 μg kg^{-1}. The LOQ for pesticides in pigeonpea matrix was 1.60 to 10.05 μg kg^{-1} considered based on the acceptable recovery (within the limit of 70–120%) for all the pesticides (Table 2). The reported LOD and LOQ values indicated the sensitivity in the method that could analyze the pesticides well below the MRLs set by the FSSAI and EU (0.01 to 2.00 mg kg^{-1}). The MRL for chlorpyrifos, imidacloprid, and lambda-cyhalothrin is 0.05 mg kg^{-1}; whereas carbaryl and carbendazim have the MRL values of 1.00 and 0.1 mg kg^{-1} by the EU and 1.50 and 0.1 mg kg^{-1}, respectively. FSSAI MRLs are also within the LOQ range of this proposed method.

Pigeonpea samples were fortified at 10, 50, and 100 μg kg^{-1} level using pesticides working standard mixture recorded 85.4 to 117.3, 84.3 to 118.8, and 85.5 to 116.2% of the recoveries, respectively with RSD less than 15% at all fortification level. Accuracy was measured by analyzing the fortified samples and comparing the measured value to the "true" value or fortified value. In this study, the accuracy for the tested pesticide was found acceptable with the criteria defined in the validation guidelines SANTAE/11813/2017. Repeatability estimation showed the recovery range of 80.20 to 109.9% with relative standard deviation in the range of 4.5 to 16.8%. The precision with intra and inter-day test with change in date of analysis and the analyst, recorded the recovery of 80.40 to 107.90 and 81.10 to 107.80%, with RSD in the range of 2.0 to 12.7%, respectively (Table 3). Results of the different parameter attended in the method fulfilled the SANTAE/11813/2017. The results of the method validation in pigeonpea matrix proved that the modified QuEChERS extraction with GC–MS/MS and LC–MS/MS detection were selective, robust, accurate, and sensitive enough for quantification of 79 different pesticides belongs to organochlorine, organophosphate and their metabolites, carbamates, synthetic pyrethroids, neonicotinoides, and others pesticides. This method could be applied to the pesticides in

Table 3
Repeatability, reproducibility and ruggedness, matrix effect, uncertainty of measurement and MRLs for multiclass pesticides and their metabolites in pigeonpea grains.

Pesticides	Repeatability		Reproducibility and Ruggedness			Matrix effect (%)	Uncertainty of Measurement (±) at 50 μg kg^{-1}		MRL (mg kg^{-1})	
	Recovery (%) @ 50 μg kg^{-1}	RSD (%)	Day 1 and Analyst 1 @ 50 μg kg^{-1}	Day 2 and Analyst 2 @ 50 μg kg^{-1}	RSD (%)		Combined Uncertainty (Uc)	Expanded Uncertainty ($U_{exp.}$)	EU	FSSAI
Dichlorvos	86.3	4.5	90.1	89.9	3.6	−3.86	3.47	6.90	–	–
Phenol, 4-bromo-2-chloro	82.6	12.3	86.6	96.6	5.2	0.64	1.71	3.42	–	–
Trifluralin	89.9	8.2	88.2	91.2	10.9	12.64	2.42	4.83	0.01	–
Alpha-BHC	95.6	10.2	80.4	91.2	5.4	−6.69	2.87	5.73	0.01	–
Beta-BHC	87.9	9.2	87.4	91.5	4.2	14.96	5.13	10.26	0.01	–
Diazinon	81.3	8.9	82.3	89.2	9.0	12.79	1.79	3.58	0.01	–
Fluchloralin	97.9	10.1	94.2	101.3	5.6	9.50	5.00	9.99	–	–
Delta-BHC	101.3	13.2	99.3	106.6	2.3	−20.94	4.38	8.75	0.01	–
Tri-allate	103.9	9.8	98.9	102.2	6.2	1.54	2.13	4.25	0.10	–
Iprobenfos	96.2	12.3	95.6	104.2	6.9	23.19	2.97	5.93	–	–
Propanil	90.2	14.2	90.0	95.6	8.0	14.55	1.67	3.34	0.01	–
Chlorpyrifos-methyl	101.4	9.8	91.2	90.0	10.2	18.80	3.88	7.75	0.05	–
Parathion-methyl	87.3	11.2	96.7	81.1	11.3	10.47	3.36	6.72	0.01	–
Alachlor	103.3	10.9	92.3	107.8	3.3	20.83	2.29	4.57	0.01	–
Heptachlor	92.3	10.6	86.6	92.1	5.6	26.11	2.56	5.11	0.01	–
Fenitrothion	97.9	11.3	102.2	94.2	4.2	21.97	5.70	11.40	0.01	–
Aldrin	96.3	10.0	81.2	93.2	7.9	8.47	4.91	9.82	0.01	–
Chlorpyrifos	85.3	10.2	92.3	86.7	2.0	14.52	4.27	8.53	0.01	0.05
Parathion	105.2	11.3	101.2	91.3	3.2	22.77	3.70	7.40	0.05	–
Chlorfenvinphos	88.3	8.6	86.2	96.2	4.3	12.92	4.44	8.87	0.01	–
Phenthoate	29.2	5.2	97.9	98.2	6.9	19.76	6.38	12.76	–	0.05
Allethrin	90.0	10.6	91.2	98.7	9.8	16.48	3.65	7.30	–	–
Alpha-Endosulfan	96.9	12.4	83.2	91.2	3.6	18.27	3.45	6.90	0.05	–
Butachlor	101.2	8.9	100.2	92.6	5.2	0.36	5.66	11.31		–
p,p'-DDE	88.9	9.9	91.2	84.2	6.7	23.88	3.59	7.18	0.05	–
Endrin	90.2	8.9	91.2	86.9	7.1	12.40	4.71	9.42	0.01	–
Beta-Endosulfan	91.2	9.2	89.0	97.1	6.6	4.09	5.28	10.56	0.05	–
p,p'-DDT	100.2	6.5	89.2	96.2	9.9	16.70	5.11	10.21	0.05	–
Endosulfansulfate	99.3	11.3	91.3	101.3	11.2	−23.14	5.47	10.94	0.05	–
o,p'-DDT	90.2	7.9	89.9	98.2	9.9	21.25	2.42	4.84	0.05	–
Bifenthrin	94.2	8.8	92.0	83.2	12.7	15.22	3.22	6.43	0.3	–
Fenpropathrin	96.3	8.9	91.3	95.7	6.9	15.83	5.21	10.42	0.01	–
L-Cyhalothrin	82.2	5.4	96.6	88.2	7.6	−11.73	3.36	6.71	–	0.05
Permethrin I	87.9	10.1	101.2	98.9	10.3	−6.86	2.70	5.40	0.05	–
Permethrin II	96.2	9.7	97.9	89.2	6.0	2.36	3.16	6.32	0.05	–
Cypermethrin	88.6	6.7	102.3	95.6	9.0	−14.21	2.24	4.47	0.05	–
Cyfluthrin	96.8	8.1	90.2	82.2	7.2	−17.13	2.43	4.85	0.02	–
Etofenprox	105.2	9.2	92.4	102.3	9.0	2.28	4.79	9.57	–	–
Fenvalerate	90.2	11.2	103.3	96.6	5.7	23.11	3.56	7.11	0.02	–
Deltamethrin	95.2	12.3	101.7	92.3	3.0	−12.61	5.06	10.11	–	0.01
Thiacloprid	106.9	16.8	90.2	104.2	5.1	−11.75	6.34	12.68	–	0.02
Buprofezin	98.3	9.8	86.7	91.2	5.4	−9.20	3.65	7.29	–	0.05
Metalachlor	99.5	11.2	85.7	80.2	6.2	−12.67	4.91	9.81	–	–
Imidacloprid	98.9	10.2	93.7	102.4	7.2	2.64	4.82	9.63	–	0.05
Dimethoate	101.9	10.2	98.9	105.5	3.9	−15.08	5.21	10.42	0.02	–
Coumatetryl	86.2	10.3	99.7	89.9	5.1	15.52	5.02	10.04	–	–
Triademenol	105.2	11.2	88.2	91.7	9.7	−3.05	3.75	7.49	–	–
Triademefon	106.6	12.9	86.2	97.2	5.5	−3.50	3.57	7.14	0.30	–
Thiobencarb	87.9	14.2	101.7	98.7	10.2	−17.46	5.48	10.96	0.30	–
Spinosad	100.0	12.7	83.3	96.7	4.9	−12.71	3.44	6.88	–	0.01
Phosalone	97.0	14.2	85.7	96.6	6.6	4.07	3.74	7.48	–	–
Methoxyfenozide	98.5	12.7	107.9	96.6	9.0	−10.54	4.31	8.62	–	–
Hexythiazox	108.2	15.6	106.7	91.2	11.2	−19.31	4.62	9.23	–	–
Fenpyroximate	109.8	9.8	99.3	102.3	8.9	1.01	4.69	9.37	–	–
Carbendazim	81.2	13.2	91.2	80.7	9.2	−10.27	3.61	7.22	0.10	0.1
Carbaryl	103.3	14.3	101.6	92.3	5.3	−4.42	5.20	10.40	1.00	1.50
Triazophos	86.7	15.6	90.2	100.3	4.9	5.06	3.82	7.63	0.02	0.60
Carbofuron	101.2	9.8	87.9	96.9	6.9	−2.64	4.90	9.80	–	0.10
Bitertenol	91.3	9.9	100.9	90.2	9.9	16.01	3.79	7.58	0.05	–
Bendiocarb	106.2	11.2	93.3	102.2	2.9	−8.89	5.34	10.68	–	–
Benalaxyl	98.2	13.3	91.2	92.7	6.4	4.38	3.69	7.37	0.05	–
Acephate	86.7	13.3	90.2	83.7	5.4	−7.27	3.79	7.57	0.02	1.00
Pymetrozine	80.2	12.2	80.1	93.2	9.7	−4.12	6.08	12.16	0.50	0.01
Omethoate	85.3	15.6	86.6	92.3	7.7	−9.37	5.93	11.86	0.20	–
Metribuzin	101.6	12.2	101.2	92.2	9.3	−10.55	5.79	11.57	–	–
Metalaxyl	99.5	16.6	90.2	86.7	6.7	−1.82	4.57	9.14	0.05	–
Emamectin benzoate	108.8	6.9	98.9	102.4	3.3	−10.51	3.50	7.00	–	–
Tetraconazole	105.6	8.0	92.2	100.9	2.9	−11.40	5.31	10.62	–	–
Quinalphos	87.6	10.2	92.2	88.2	5.3	−8.01	4.62	9.24	0.05	0.01

(*continued on next page*)

Table 3 (*continued*)

Pesticides	Repeatability		Reproducibility and Ruggedness			Matrix effect (%)	Uncertainty of Measurement (±) at 50 μg kg^{-1}		MRL (mg kg^{-1})	
	Recovery (%) @ 50 μg kg^{-1}	RSD (%)	Day 1 and Analyst 1 @ 50 μg kg^{-1}	Day 2 and Analyst 2 @ 50 μg kg^{-1}	RSD (%)		Combined Uncertainty (Uc)	Expanded Uncertainty ($U_{exp.}$)	EU	FSSAI
Profenofos	88.2	10.1	96.2	83.4	11.3	−7.56	3.76	7.51	–	–
Phosphomidon	106.6	9.0	102.3	98.6	7.0	−2.88	5.57	11.14	–	–
Pendimethalin	109.9	16.8	89.3	96.7	7.3	1.22	6.24	12.47	–	0.05
Difenconazole	99.6	11.2	88.7	97.9	4.5	4.78	3.60	7.20	–	–
Pretilachlor	87.8	12.2	93.4	103.4	5.2	−17.91	3.66	7.32	–	0.05
Paclobutrazole	93.6	13.5	91.3	81.2	6.0	−11.82	4.28	8.55	–	–
Chlorantraniliprole	101.3	16.0	93.9	101.0	8.9	−12.36	4.97	9.94	–	0.03
Isoproturon	98.9	13.3	98.2	104.2	5.2	−4.41	4.02	8.03	0.05	–
Hexaconazole	100.3	8.9	97.9	103.4	4.0	8.34	4.39	8.78	0.10	0.02
Penconazole	105.3	10.2	98.3	87.9	11.2	−9.46	3.76	7.51	0.05	–

RSD-Relative standard Deviation; EU-European Union; FSSAI-Food Safety Standard Authority of India; MRL-Maximum Residue Level/Limit.

Table 4
Pesticide residues detected in pigeonpea real (field) samples (n = 50) collected from farmers field.

Pesticide	Residue (mg kg^{-1})	MRL (mg kg^{-1})		Confirmation technique
		FSSAI	EU	
Profenofos	0.015–0.034	NA	0.05	LC–MSMS
Lambda-cyhalothrin	0.010–0.024	0.05	0.05	GC–MSMS
Chlorpyrifos	0.015–0.068	0.05	0.01	GC–MSMS
Quinalphos	BQL-0.007	0.01	0.05	LC–MSMS
Permethrin	0.010–0.038	NA	0.05	GC–MSMS
Deltamethrin	0.010–0.014	NA	0.01	GC–MSMS
Dimethoate	BQL-0.010	NA	0.02	LC–MSMS
Imidacloprid	BQL-0.010	NA	0.05	LC–MSMS
Fenpropathrin	BQL-0.011	NA	0.01	GC–MSMS

BQL: Below Quantification limit; MRL: Maximum Residue Level; FSSAI: Food Safety Standard Authority of India; EU: European Union; NA: Not Available.

field-collected pigeonpea samples for judging their quality in terms of the pesticides free harvest and the estimation of pesticides in the check samples received under proficiency testing program.

3.4. Matrix effect

A higher and lower slope in the matrix calibration indicated matrix-induced signal enhancement and suppression, respectively. Matrix effects were in the range of −23.14 to 26.11% (Table 2), which was above the maximum threshold limit of 20% for some pesticides (European Commission, 2017). Among 79 pesticides, 40 pesticides have a positive effect, and the rest reported negative values to represent matrix-induced suppression. Therefore, for the quantification of pesticides, it is essential to use a matrix-matched calibration response for obtaining accurate quantification results for test analytes or pesticides. This was supported by the earlier research for quantitation of residues based on matrix-matched calibration to compensate for the matrix effect and obtain more accurate results for the pesticides studied (da Silva et al., 2012). Overall, the matrix effect was not affected by the realistic concentration of the pesticide and its metabolites in this study (Table 2).

3.5. Estimation of measurement of uncertainty

The measurement uncertainty with the method was calculated as standard uncertainty, which is occurred due to the sample recovery upon spiking with a particular concentration of pesticide mixture. It was found in the range of 3.42 to 12.76 μg kg^{-1} tested at 50 μg kg^{-1} fortification level in the pigeonpea matrix (Table 2). The uncertainty caused due to the various other sample processing steps involved is called type B uncertainty. Then combined uncertainty was evaluated considering all the factors as the contributions were independent of each other. The uncertainty values reported with the method for pigeonpea matrix was found acceptable. This indicated that the developed method was highly sensitive and suitable for -multiclass pesticide analysis in pigeonpea grain. A method standardized for analysis of pesticide residues in spent leaves, made tea and tea infusion evaluated for their uncertainty values found acceptable for most of the pesticides indicating the method reliable and highly sensitive and suitable (Banerjee et al., 2007; Kanrar et al., 2010). The estimation of measurement uncertainty with a single matrix lot is the common approach in quality control laboratories for routine analysis. In the previous study, different lots of maize and fig matrix contributed the variation in (lot-to-lot variation) uncertainty of measurement for aflatoxin. The estimation of uncertainty of measurement in different matrix lots leads to a more realistic estimate of uncertainty of measurement (Stadler, Sulyok, Schuhmacher, Berthiller, & Krska, 2018) which can be followed in contaminant analysis and declaring the results for a sample.

3.6. Proficiency test

A pulses (chickpea) sample was anlysed using the proposed method. The validation guidelines clearly state that the validated method can be employed for analysis of commodity group and representative commodity. In this study, the sample found detected with fenvalerate (1.67), lambda-cyhalothrin (1.25), and carbaryl (0.91). The Z score for the chemical pesticides reported in the sample was found acceptable within the statistical value of −2 to +2 (Table S2). It is important to understand the statistical limitations of quality assessment through external means when evaluating the laboratory's technical competence in terms of analytical results reported using the methods standardized.

3.7. Detection of pesticide residue in real samples

Pesticide such as profenofos, lambda-cyhalothrin, chlorpyrifos, quinalphos, permethrin, deltamethrin, dimethoate, imidacloprid, fenpropathrin were commonly detected and quantified pesticides in the pigeonpea grain samples drawn from farmers' field. The residue level was in the range of 0.010 to 0.068 mg kg^{-1}. Most of these detected pesticides were found below the MRLs fixed by FSSAI and European Union except three pesticides. The pesticides could detect at a very low level by this proposed method in the pigeonpea grains collected from farmers' fields. The residues of lambda-cyhalothrin, chlorpyrifos, and deltamethrin found above the MRLs (Table 4). Photo-degradation is the major breakdown process of pesticides under natural conditions. The pigeonpea has grown in India under a tropical climate, and the grains were allowed to dry in the field after attaining maturity may be the reason for low residues. In a similar study on simultaneously determine

52 pesticides in medicine and food, dual-purpose herbs detected with monocrotophos, carbofuran, endrin, metalaxyl, *p,p*-DDT, and lambda-cyhalothrin (Gang et al., 2012) and a pesticide (dichlorvas) were quantified at 9.58 μg kg^1 in the real sample using the method developed for the analysis of 200 pesticides using GC–MS/MS (He et al., 2015) were some of the studied pesticides in the present method. The relative matrix effect (intra-matrix variation) estimation recorded no intra-matrix variation among different samples of pigeonpea collected from the farmers' field and found an acceptable deviation from a nominal value expressed as a percentage RSD_{RME} (Relative Standard Deviation for Relative Matrix Effect) of ≤15% (Steiner et al., 2020).

4. Conclusions

In this paper, LC and GC tandem mass spectrometry analytical method was developed and validated for the simultaneous determination of multi-class pesticides in pigeonpea grains. Recoveries of target pesticides were in the range 70–120%. with satisfactory precision (RSD < 20%) and can detect and quantify the pesticide residues below 10 μg $kg^{-1,}$ which complies with the MRLs set for pigeonpea grains. The method developed was suitable for analyzing routine monitoring grain samples and regulatory samples as the estimated measurement uncertainty (U_x) was a range of 3.42 to 12.76 μg kg^{-1} evaluated at 50 μg kg^{-1}. GC–MS/MS and LC–MS/MS-based methods are most essential to detect the pesticides at an extremely lower level (lower than MRLs) and can be employed for continuous monitoring of pesticides, of which most of them are currently being used in food grain production to avoid the potential risk to the consumers.

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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.

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Appendix A. Supplementary data

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