



# Validation of a modified QuEChERS method to determine multiclass multipesticide residues in apple, banana and guava using GC–MS and LC–MS/MS and its application in real sample analysis

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Received: 14 November 2019 / Accepted: 6 January 2020 / Published online: 10 January 2020  
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## Abstract

A modified QuEChERS method was developed for the determination of 64 pesticides in apple, guava and banana using GC–MS and LC–MS/MS. Out of sixty-four pesticides, 39 (32 insecticides + 7 herbicides) were analysed by GC–MS and others were analysed in LC–MS/MS. The efficiency of methanol, ethyl-acetate and acetonitrile as extracting solvents were checked. Among these, acetonitrile gave the highest recovery. d-SPE clean-up was done for apple and guava using PSA, whereas PSA + GCB was used for banana to remove heteropolysaccharides and carotenoids. The method was validated as per SANTE/11813/2017 guidelines. The LOD was in the range of 0.002–0.04 mg kg<sup>-1</sup> and LOQ was 0.005–0.11 mg kg<sup>-1</sup>. Average recovery ranged from 80 to 120% with RSD ≤ 20% and RPD % of precision ≤ 20%. The expanded uncertainty was ≤ 20%. The validated method was applied to check the real samples of apple, guava and banana collected from markets of four different districts of West Bengal, India.

**Keywords** QuEChERS · Pesticides · Apple · Guava · Banana · LC/MS/MS · GC/MS

## 1 Introduction

The nutritional intake from fruits and vegetables is higher among urban population than that of rural population. Along with the urbanisation, people are likely to increase their calorie intake at a higher pace through fruits and vegetables [1]. Apple, banana, guava are the most produced and consumed fruits around the world. India was the highest producer of guava and banana and fifth highest producer of apple around the world [2]. During 2018–2019, India exported fruits and vegetables worth Rs. 10236.93 crores/1,469.33 USD Millions which comprised of fruits worth Rs. 4817.35 crores/692.01 USD Millions and vegetables worth Rs. 5419.48 crores/777.25 USD Millions [3]. During cultivation in Indian climatic condition, fruits suffer a lot of problem due to attack of pest and diseases that would

make yield reduction as well as downturn the food quality. The use of pesticides therefore is becoming necessary and the residues of which are coming in food matrices with an elevated amount.

The wide-range use of pesticides and their highly persistent nature are the major affair for the availability of the residue in environment and food stuffs. The presence of residue in food with high level to the consumers is the thing of global concern. Due to this fact, these pesticides must be documented and precisely scanned. Several methods have been reported for the analysis of multiclass pesticide residues in fruit matrices, associating soxhlet extraction [4], liquid–liquid extraction (LLE) [5], matrix solid phase dispersion (MSPD) [6], microwave assisted extraction (MAE) [7], accelerated solvent extraction (ASE) [8], ultrasound extraction [9] and solid phase

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extraction (SPE) [10, 11]. These methods have been used with limited success. To optimize and detect the pesticides, therefore a simple, rapid and cost-effective method is needed. Anastassiades and his co-workers [12] developed a multi residue method for the analysis of pesticide residue from food samples, known as QuEChERS. Considering the fact, a modified QuEChERS method [13] have been developed to detect residues of multipesticides using gas chromatography coupled with single quadrupole and liquid chromatography associated with triple quadrupole, without any hint on cleaning up of banana matrix. A total of sixty-four pesticides were chosen based on the report that apple, guava and banana growing farmers are usually spraying these pesticides in India. It is important to monitor residue levels of these pesticides in these raw consumable fruits. Based on the polarities of the pesticides GC-MS and LC-MS/MS were chosen. Out of 64 pesticides, 39 (= 31 insecticides including OCs, OPs and synthetic pyrethroids + 1 tetroneic acid insecticide spiromesifen + 7 herbicides) pesticides were analysed with GC-MS and 25 pesticides were analysed with LC-MS/MS. The purpose of the study is to develop multi-class, multi-residue method based on QuEChERS for the analysis of above-mentioned pesticides which are frequently used in apple, guava and banana orchards. Also, the method shall assist to detect and determine the quantities of pesticide loads for monitoring of apple, guava and banana fruit meant for export.

## 2 Materials and methods

### 2.1 CRMs, chemicals and apparatus

All CRMs of 64 pesticides with purity  $\geq 98\%$  were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Individual mother stock solutions ( $500 \text{ mg L}^{-1}$ ) of 39 GC amenable pesticides were prepared with hexane-toluene (1:1) mixture and that of 25 LC amenable pesticides were prepared in acetonitrile. Intermediate stock solution ( $100 \text{ mg L}^{-1}$ ) were prepared respectively in the same solvents, by appropriate dilution from the mother stock solution and were stored at  $4^\circ\text{C}$ . To prepare  $10 \text{ mg L}^{-1}$  mixture of 64 pesticides, the required volumes of intermediate stock solution of pesticides were added and hexane-toluene mixture, acetonitrile were evaporated in Turbo-vap. The final volume was made with acetonitrile. The working standard solutions of 0.01, 0.05, 0.1, 0.5 and  $1 \text{ mg L}^{-1}$  prepared in acetonitrile from  $10 \text{ mg L}^{-1}$  for LC-MS/MS. Acetonitrile from the respective working standard solutions were evaporated up and reconstituted in hexane for GC-MS. Matrix-matched standards of 0.01, 0.05, 0.1, 0.5 and  $1.0 \text{ mg L}^{-1}$  prepared by evaporating appropriate volumes of a standard mixture solution and diluting with respective matrix of apple, guava

and banana in hexane and acetonitrile respectively for use in GC-MS and LC-MS/MS. The working standard solutions were also stored at  $4^\circ\text{C}$ . Analytical grade NaCl and anhydrous  $\text{MgSO}_4$  were obtained from Merck, Darmstadt, Germany. The  $\text{MgSO}_4$  was baked for 5 h at  $500^\circ\text{C}$  in a muffle furnace to remove phthalates. Primary Secondary Amine (PSA) and Graphitized Carbon Black (GCB) sorbent were purchased from Agilent Technology, USA. Ammonium formate buffer (Bio-ultra  $\geq 99.0\%$ ) was procured from Fluka Milan, Italy. Analytical LC-MS grade acetonitrile, hexane, acetone, water, methanol and toluene were obtained from J.T. Baker, Avantor, USA. High precision calibrated analytical balance (Sartorius AG, Göttingen, Germany) was used for weighing the CRMs, reagents and samples accurately. A Robot Coupe Blixer 6V.V (7L) Vincennes, France was used to comminute the fruit samples. A vortex mixer (Spinix, Tarsons, Kolkata, India), Rotospin (Tarsons, Kolkata, India), Silent Crusher (Heidolph, Schwabach, Germany), Centrifuge (Super Spin R-V/FM Plasto Crafts, Mumbai, India) and Turbo Vap evaporator (Caliper Life Sciences, Hopkinton, Massachusetts, USA) were used for sample preparation. A solvent filtration unit (Borosil, India) and micropipettes (Boeco, Germany) of  $1000 \mu\text{l}$ ,  $5000 \mu\text{l}$  and  $10 \text{ ml}$ , were used.

### 2.2 Selection of pesticides and fruits

Apple, guava and banana were selected for the study based on their export potential. All these sixty-four pesticides (50 insecticides, 8 fungicides and 6 herbicides) were selected for our study based on the report that farmers are using these pesticides in apple, guava and banana in India. Therefore, it is necessary to monitor their residue levels in raw consumable fruits. Apple (*Malus domestica* var. *Golden delicious*), Banana (*Musa acuminata* var. *Singapuri*) and Guava (*Psidium guajava* var. *Khaja*) were randomly collected from the untreated control plots of research trials. These fruits were screened and confirmed that no any pesticide was present before using these in the method developing process. Real samples of apple, guava and banana were collected from the markets of Kolkata, Howrah, Hooghly and Burdwan districts of west Bengal, India. The samples were well comminuted, and placed in glass bottles, and stored at  $-20^\circ\text{C}$ .

### 2.3 Instrumentation

GCMS-QP 2010 Plus (Shimadzu Corporation, Kyoto, Japan) with a mass selective detector (MSD, single quadrupole) and a Capillary column DB-5MS J&W  $30 \text{ m} \times 0.25 \text{ mm}$  id  $\times 0.25 \mu\text{m}$  (Agilent, USA,) was used for confirmation and quantification study. GC-MS operated under following GC conditions: Initial temperature  $40^\circ\text{C}$  for 1 min, then increased @  $25^\circ\text{C min}^{-1}$  to  $130^\circ\text{C}$  and @  $12^\circ\text{C min}^{-1}$

to 180 °C and again increased @3°Cmin<sup>-1</sup> to 280 °C, then hold for 7 min. Injector temperature was 250 °C. Carrier gas used was Helium (purity 99.999%). Ion source temperature was 250 °C. Interface temperature was 280 °C. The instrument operated in the split mode with split ratio 1:10. Purge flow was 3 ml min<sup>-1</sup>. Injection volume was 2 µL. MS condition were as follows: delay solvent was 6 min;

electron impact ionization voltage was 70 eV; scan rate was 0.50 s<sup>-1</sup>; scanned mass ranged 50–500 m/z. All samples were analysed in the Selected Ion Monitoring (SIM) mode. Retention times, selected monitoring ions used for the identification and confirmation are depicted in Table 1. The LC–MS/MS analysis was carried out using an API-3200 LC–MS/MS system (AB Sciex, Vaughan, Canada)

**Table 1** Optimization of different parameters of 39 pesticide CRMs in GC–MS

Pesticide	GC–MS, SIM (min.)					
	Retention Time (min.)	Time (min.)		m/z for confirmation with ion ratio		
		Start	End	Target (T)	Q <sub>1</sub> (%Q <sub>1</sub> /T)	Q <sub>2</sub> (%Q <sub>2</sub> /T)
4-Br-2Cl- phenol	8.96	8.44	9.14	208	172 (42.09%)	170 (32.58%)
Trifluralin	13.61	13.574	13.79	306	43 (44.72%)	264 (40.28%)
Phorate	14.31	14.30	14.43	75	121 (50.28%)	260 (43.29%)
Alpha-HCH	14.59	14.43	14.71	181	183 (93.37%)	219 (51.04%)
Dimethoate	14.96	14.81	15.12	87	93 (51.23%)	125 (42.30%)
Atrazine	15.35	15.08	15.59	200	215 (97.23%)	58 (76.68%)
Beta-HCH	15.52	15.49	15.87	181	183 (68.78%)	219 (95.78%)
Lindane	15.76	15.59	15.87	181	183 (96.48%)	219(54.23%)
Phosphamedon	16.03	15.91	16.13	127	72 (40.28%)	264 (40.89%)
Chlorothalonil	16.38	15.87	16.43	266	264 (76.28%)	268 (50.23%)
Delta-HCH	16.92	16.83	17.04	181	183 (92.49%)	219(57.85%)
Dimethachlor	17.76	17.69	17.86	134	197 (40.23%)	77 (52.91%)
Parathion-methyl	18.31	18.25	18.45	263	109 (42.21%)	125 (35.23%)
Alachlor	18.30	18.22	18.45	45	160 (42.28%)	188 (26.22%)
Heptachlor	18.71	18.65	18.84	100	272 (42.28%)	274 (30.56%)
Malathion	19.80	19.71	19.92	125	127 (65.59%)	93 (47.54%)
Chlorpyrifos	20.14	19.92	20.23	97	197 (63.28%)	199 (41.56%)
Aldrin	20.36	20.23	20.50	66	263 (57.63%)	91 (22.73%)
Pendimethylene	21.77	21.50	21.90	252	162 (18.93%)	181 (20.43%)
Quinalphos	22.85	21.70	22.96	146	118 (65.00%)	156 (61%)
OP-DDD	23.72	23.62	23.80	235	165 (76.43%)	237 (53.85%)
Butachlor	23.87	23.70	24.02	57	176 (68.89%)	160 (76.31%)
Alpha-endo	24.18	24.02	24.46	241	195 (80.25%)	159 (22.91%)
PP-DDD	24.91	24.56	25.05	235	165 (56.28%)	237 (32.50%)
Profenophos	25.20	25.10	25.35	337	97 (68.11%)	139 (66.97%)
PP-DDE	25.39	25.20	24.56	246	318 (75.76%)	248 (54.81%)
OP-DDT	25.75	25.58	25.82	235	165 (40.12%)	237 (32.20%)
Beta-Endosulfan	27.42	27.25	27.52	241	195 (65.23%)	159 (40.21%)
Ethion	27.77	27.62	27.80	231	97 (60.28%)	153 (50.78%)
PP-DDT	27.77	27.62	27.80	235	165 (52.28%)	237 (61.12%)
Endo sulphate	29.67	29.52	29.95	272	274 (53.37%)	239 (50.21%)
Spiromesifen	31.70	31.55	31.95	272	99 (52.23%)	273 (36.16%)
Bifenthrin	32.91	32.74	33.12	181	166 (53.27%)	165 (62.74%)
Fenprothrin	33.45	33.20	33.70	97	181 (96.97%)	265 (35.12%)
Lamda-cyhalothrin	36.23	36.14	36.30	181	197 (95.70%)	208 (58.77%)
Beta-cyfluthrin	41.24	41.07	41.40	163	206 (65.23%)	226 (54.15%)
Cypermethrin	42.24	42.10	42.30	181	163 (75.86%)	127 (47.59%)
Fenvalerate	44.76	44.60	44.92	125	167 (57.88%)	225 (44.54%)
Deltamethrin	47.87	47.72	47.99	181	253 (72.62%)	77 (69.84%)

C.R.M., certified reference materials; R.T., retention time; SIM., selected ion monitoring

hyphenated to a Waters UPLC (USA) controlled by Analyst 1.5 software. The chromatographic separation was carried out injecting 10 µL onto a reverse phase Zorbax SB-C18 (4.6 mm × 150mm × 5 µm) column (Agilent Technologies, USA) maintained at 35 °C with mobile phase flow rate of 0.35 ml min<sup>-1</sup>. The mobile phase was composed of (A) 5 mM ammonium formate in methanol and (B) 5 mM ammonium formate in water. The gradient elution programme was as follows: A (95%) B (5%) at the initial time (0 min), A (95%) B (5%) (at 1.70 min), A (50%) B (50%) (at 4.90 min), A (10%) B (90%) (at 9.90 min), A (10%) B (90%) (at 11.50 min), A (95%) B (5%) (at 13.21 min), A (95%) B (5%) (at 14 min). Total run time was 14 min. The mass spectrometric analysis of all 25 pesticides performed were estimated in positive electrospray ionization mode [ESI (+ve)] with dwell time of 30 ms. Here using two abundant precursor/products were used for transition of ion in MS/MS analysis for the construction of MRM. The MS source condition was: The ion source temperature was set at 500 °C, ion spray voltage was 5500 V in positive mode. Curtain gas of 30 psi, collisionally activated dissociation gas (CAD) of 5 psi,

nebulizer gas (GS1) of 40 psi, heater gas of (GS2) 40 psi were used. The specific mass spectrometric parameters of 25 compounds were given in Table 2. The identification and quantification were performed in samples and standards in accordance with the confirmation criteria of the EC guidelines.

## 2.4 Sample preparation

About 2 kg of fruit (apple, banana, guava) samples were collected from the untreated control plots of research trials and chopped sample was homogenized in Robot Coupe Blixer @5000 rpm for 10 min. The well comminuted samples were placed in glass bottles (Tarson make) and stored at -20 °C before analysis. For each substrate, 15 g homogenized sample was taken into three 50 ml centrifuge tubes to optimize the extraction procedure and 15 ml three different solvents i.e. methanol, ethyl-acetate and acetonitrile were added. Then vortexed for 1 min. The mixture was then homogenized by Silent Crusher @ 12,500 rpm for 1 min. 1.5 g of activated sodium chloride

**Table 2** Optimization of instrument acquisition parameters of 25 pesticide CRMs in LC-MS/MS

Pesticides	RT (min)	Precursor ion (m/z)	First Transition quantification			Second transition identification		
			Product ion (m/z)	DP(V)	CE(V)	Product ion (m/z)	DP(V)	CE(V)
Carbendazim	5.31	19	160	33	30	132	33	43
Imidacloprid	4.88	256	209	41	21	175	41	31
Thiacloprid	5.01	253	126	61	25	90	61	55
Carbofuran	5.32	222	165	61	23	123	61	29
Carbaryl	5.36	202	145	31	17	127	31	39
Triazophos	5.88	314	162	29	25	119	29	49
Monocrotophos	4.76	224	127	31	21	98	31	17
Spirotetramet	6.04	374.40	302.40	56	23	330.50	46	23
Tebuconazole	6.54	308	70	61	55	125	61	59
Hexaconazole	6.96	314	70	52	38	159	52	38
Anilophos	6.30	368	199	55	20	171	55	29
Thiamethoxam	4.73	292	211	46	19	181	46	31
Acetamiprid	4.88	223	126	60	27	56	60	35
Chlorfenvinphos	6.46	359	99	55	49	170	55	66
Propiconazole	6.96	342	159	30	33	69	30	40
Fenamidon	5.59	312	236	53	21	92	53	35
Phosphamidon	5.02	300	174	49	34	132	49	34
Dimethoate	4.90	230	171	30	21	199	30	14
Indoxacarb	6.21	528	203	81	56	249	81	25
Omethoate	4.64	214	125	45	35	109	45	42
Methomyl	4.92	163	106	34	70	88	34	17
Choranthraniliprole	5.34	482	451	68	28	283	68	25
Edifenphos	6.67	311	283	61	20	109	61	46
Thiodicarb	5.34	355.20	88	55	25	108	55	20
Trifloxystrobin	6.57	409	186	47	26	186	47	22

D.P. deculsting potential, E.P. entrance potential

and 4 g anhydrous phthalate free  $MgSO_4$  were added to it. Reagents were thoroughly mixed by vortex mixer for 1 min and rotospined for 5 min and then centrifuged for 5 min @ 10,000 rpm speed. In the d-SPE clean-up step, 5 ml supernatant was transferred to each of the 15 ml centrifuge tubes containing 250 mg PSA sorbent, 25 mg GCB (for banana only) and 750 mg anhydrous  $MgSO_4$ . The tube was capped, vortexed for 30 s and centrifuged for 5 min @ 5000 rpm speed. 1 ml supernatant was transferred into a vial for LC-MS/MS analysis and another portion was taken in a tube and solvent was evaporated by Turbovap evaporator at 40 °C and dried under a gentle stream of pure nitrogen. Finally, the residue was reconstituted in 1 ml acetone and filtered through 0.2  $\mu m$  ultipore nylon 6,6 membranes for GC-MS analysis. The same method was applied to the fortified and real samples.

## 2.5 Validation as per Eurachem and SANTE guidelines

The developed analytical method was validated with linearity, precision, accuracy, LOD, LOQ and measurement of uncertainty as per the recommendations of Eurachem [14] and SANTE guidelines [15]. LOD and LOQ were determined based on signal to noise ratio (S: N) 3:1 and 10:1 respectively. The five-point (0.01-1.0 mg  $kg^{-1}$ ) calibration curve was prepared for checking linearity with the regression coefficient ( $R^2$ ) for both pure and matrix-matched standard mixtures. Recovery experiment in seven replicates was carried out by fortifying 15 gm homogenized fruit (apple, banana and guava) sample @ LOQ, 5  $\times$  LOQ and 10  $\times$  LOQ level (Table 3). Pesticides having similar LOQ as well as MRL values were fortified at LOQ/MRL level. Fortifications were done with the pure working standards. The quantification was done by matrix-matched standard to appraise the robustness of the method. The precision of the method was evaluated by two steps (a) intra-day (repeatability) which is the analysis of the sample in five replicates in one day by one analyst and their value is calculated by percentage of relative standard deviation, (b) inter-day (reproducibility) analysis was done by two analysts with the same sample (five replicates) in two alternative days by following all the same experimental and instrumental conditions. The results of precision reproducibility are also calculated with the %RSD. Precision actually asserted the trueness of the method by expressing the value as relative percentage deviation called %RPD [16] @ LOQ level that can be represented by the following equation:

Relative percentage deviation (%RPD)

$$= \left[ \frac{(\text{Interday recovery} - \text{Intraday recovery})}{(\text{Interday recovery} + \text{Intraday recovery})/2} \right] \times 100$$

Matrix effect can be evaluated by the following equation:

$$\%ME = (F - 1) \times 100$$

$F = S_{\text{matrix}}/S_{\text{standard}}$ , where,  $S_{\text{matrix}}$  represents peak area of the fortified extract and  $S_{\text{standard}}$  states peak area of the pure standard. Positive value of %ME indicates matrix enhancement and negative value will be matrix suppression. For the strong matrix effect  $\%ME > 50$ ,  $20 < \%ME \leq 50$  having considered the medium matrix effect [17]. But currently  $\%ME \leq 20$  (enhancement or suppression) as per SANTE guideline is accepted. For the uncertainty measurement top down approach was used. The combined uncertainty that is associated with standard and sample was calculated as per the following equation:

$$U_c = \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 + U_5^2 + U_6^2 + U_7^2 + U_8^2 + U_9^2 + U_{10}^2 + U_{11}^2)}$$

Where,  $U_1$ = recovery-accuracy,  $U_2$ = recovery-precision,  $U_3$ =purity of standard,  $U_4$ =balance for standard preparation,  $U_5$ =volumetric flask for stock solution,  $U_6$ =volumetric flask for working standard solution,  $U_7$ = 1 ml pipette,  $U_8$ =5 ml pipette,  $U_9$ =linearity of balance,  $U_{10}$ =uncertainty of seven points calibration,  $U_{11}$ =uncertainty precision of instrument at LOQ. Expanded uncertainty (U) is twice of combined uncertainty at a confidence level of 95%.

$$\text{Total uncertainty (MU)} = \text{LOQ} \times 2 U_c$$

## 3 Results and Discussion

### 3.1 Standardization of Extraction and cleaning up step

Carneiro and his co-workers [13] reported extraction process by modified QuEChERS but they did not use any clean-up step for banana matrix. Jardim and her team [18] used buffer QuEChERS in apple and guava samples. In the present study, extracting solvent was standardized, among three different extracting solvents i.e., methanol, acetonitrile and ethyl-acetate used based on their polarities. A comparative picture of average percent recovery using these extracting solvents which provided the representative data was presented in Fig. 1. Based on the data, acetonitrile was standardised as the extracting solvent

**Table 3** Average % recovery ( $\pm$  RSD) (n = 7) of 64 pesticides in apple, banana, guava fortified @ LOQ, 5 x LOQ and 10 x LOQ level along with %ME and %uncertainty (k = 2) when analysed in GC-MS and LC-MS/MS

Pesticides	Fortification (mg kg <sup>-1</sup> ) LOQ	Average % recovery $\pm$ RSD		Fortification 5xLOQ (mg kg <sup>-1</sup> )	Average % recovery $\pm$ RSD		
		Apple	Banana		Apple	Banana	
<i>GC-MS amenable pesticides</i>							
4-Br-2Cl-phenol	0.05	106 $\pm$ 7.69	100 $\pm$ 8.22	0.25	103 $\pm$ 5.45	104 $\pm$ 8.97	92 $\pm$ 2.11
Trifluralin	0.05	99 $\pm$ 0.47	99 $\pm$ 16.88	0.25	88 $\pm$ 1.56	97 $\pm$ 6.02	96 $\pm$ 3.51
Phorate	0.05*	90 $\pm$ 0.99	106 $\pm$ 7.39	0.25	107 $\pm$ 8.59	97 $\pm$ 8.57	100 $\pm$ 5.79
alpha-HCH	0.01#	86 $\pm$ 3.68	102 $\pm$ 7.22	0.05	96 $\pm$ 4.82	102 $\pm$ 11.37	97 $\pm$ 9.50
Dimethoate	0.05+	111 $\pm$ 9.60	98 $\pm$ 7.23	0.25	102 $\pm$ 9.90	90 $\pm$ 4.79	108 $\pm$ 8.25
Atrazine	0.05	111 $\pm$ 9.60	88 $\pm$ 3.89	0.25	99 $\pm$ 9.98	97 $\pm$ 7.27	91 $\pm$ 2.38
beta-HCH	0.01#	102 $\pm$ 0.83	96 $\pm$ 7.23	0.05	86 $\pm$ 3.00	92 $\pm$ 4.15	103 $\pm$ 6.88
Lindane	0.01#	102 $\pm$ 0.84	108 $\pm$ 8.56	0.05	100 $\pm$ 2.84	92 $\pm$ 13.09	109 $\pm$ 2.63
Phosphamidon	0.05\$	102 $\pm$ 1.80	92 $\pm$ 14.34	0.25	110 $\pm$ 13.62	95 $\pm$ 8.29	108 $\pm$ 2.85
Chlorothalonil	0.01	108 $\pm$ 6.33	111 $\pm$ 5.76	0.05	105 $\pm$ 8.65	89 $\pm$ 7.05	110 $\pm$ 6.75
delta-HCH	0.01#	101 $\pm$ 3.47	91 $\pm$ 10.06	0.05	109 $\pm$ 5.17	100 $\pm$ 17.28	92 $\pm$ 5.84
Dimethachlor	0.05	102 $\pm$ 1.80	96 $\pm$ 10.20	0.25	92 $\pm$ 6.86	109 $\pm$ 12.35	109 $\pm$ 4.56
Alachlor	0.05	97 $\pm$ 5.15	109 $\pm$ 11.24	0.25	104 $\pm$ 0.96	102 $\pm$ 6.97	111 $\pm$ 6.64
Parathion-methyl	0.05\$	103 $\pm$ 3.28	102 $\pm$ 13.55	0.25	99 $\pm$ 8.40	88 $\pm$ 8.21	88 $\pm$ 12.93
Heptachlor	0.05	96 $\pm$ 3.30	108 $\pm$ 7.56	0.25	103 $\pm$ 2.98	94 $\pm$ 10.96	103 $\pm$ 4.24
Malathion	0.05 <sup>δ</sup>	104 $\pm$ 5.60	95 $\pm$ 10.25	0.25	96 $\pm$ 8.11	101 $\pm$ 10.129	98 $\pm$ 5.52
Chlorpyrifos	0.05 <sup>β</sup>	101 $\pm$ 1.34	104 $\pm$ 9.80	0.25	102 $\pm$ 6.62	98 $\pm$ 5.30	98 $\pm$ 2.28
Aldrin	0.05 <sup>θ</sup>	103 $\pm$ 2.23	109 $\pm$ 10.07	0.25	103 $\pm$ 3.11	87 $\pm$ 3.07	105 $\pm$ 6.04
Pendimethylene	0.05	99 $\pm$ 0.92	102 $\pm$ 10.28	0.25	107 $\pm$ 2.20	107 $\pm$ 11.61	113 $\pm$ 3.14
Quinalphos	0.05	105 $\pm$ 4.58	98 $\pm$ 4.46	0.25	104 $\pm$ 10.27	106 $\pm$ 10.86	97 $\pm$ 4.60
op-DDD	0.01	100 $\pm$ 1.55	101 $\pm$ 6.13	0.05	89 $\pm$ 3.67	109 $\pm$ 11.81	105 $\pm$ 10.48
Butachlor	0.05	100 $\pm$ 1.06	109 $\pm$ 7.51	0.25	103 $\pm$ 1.86	102 $\pm$ 6.55	86 $\pm$ 2.54
alpha-endosulfan	0.01 <sup>+</sup>	100 $\pm$ 0.61	104 $\pm$ 7.04	0.05	111 $\pm$ 7.37	95 $\pm$ 6.16	96 $\pm$ 5.51
pp-DDD	0.01 <sup>£</sup>	96 $\pm$ 4.63	99 $\pm$ 1.72	0.05	100 $\pm$ 5.84	96 $\pm$ 2.26	117 $\pm$ 2.06
Profenophos	0.05	109 $\pm$ 6.33	103 $\pm$ 12.38	0.25	90 $\pm$ 8.74	95 $\pm$ 12.43	97 $\pm$ 4.91
pp-DDE	0.01 <sup>£</sup>	99 $\pm$ 0.67	110 $\pm$ 7.82	0.05	100 $\pm$ 8.31	116 $\pm$ 5.90	99 $\pm$ 2.53
op-DDT	0.01 <sup>£</sup>	105 $\pm$ 13.45	111 $\pm$ 12.86	0.05	101 $\pm$ 12.36	114 $\pm$ 6.516	113 $\pm$ 8.05
beta-endosulfan	0.01 <sup>+</sup>	96 $\pm$ 4.63	90 $\pm$ 5.89	0.05	100 $\pm$ 5.84	93 $\pm$ 3.75	96 $\pm$ 7.52
Ethion	0.05 <sup>+</sup>	101 $\pm$ 3.09	104 $\pm$ 10.12	0.25	103 $\pm$ 2.42	105 $\pm$ 13.19	85 $\pm$ 4.84
pp-DDT	0.01 <sup>£</sup>	107 $\pm$ 13.91	87 $\pm$ 9.75	0.05	113 $\pm$ 4.27	94 $\pm$ 8.95	108 $\pm$ 5.59
Endosulfan sulphate	0.01 <sup>+</sup>	99 $\pm$ 9.25	115 $\pm$ 6.99	0.05	102 $\pm$ 6.30	92 $\pm$ 10.78	99 $\pm$ 8.23
Spiromesifen	0.1	106 $\pm$ 2.57	96 $\pm$ 10.14	0.5	103 $\pm$ 5.61	97 $\pm$ 15.41	109 $\pm$ 1.52

Table 3 (continued)

Pesticides	Fortification (mg kg <sup>-1</sup> ) LOQ	Average % recovery ± RSD			Fortification 5xLOQ (mg kg <sup>-1</sup> )	Average % recovery ± RSD		
		Apple	Guava	Banana		Apple	Guava	Banana
Bifenthrin	0.1	102±3.30	88±4.43	103±15.13	0.5	106±4.44	87±8.09	110±1.38
Fenpropathrin	0.1	107±2.87	88±4.01	110±11.01	0.5	90±4.54	103±13.00	113±2.94
lamda cyhalothrin	0.1	103±2.23	93±7.21	92±10.90	0.5	101±4.20	87±4.03	89±1.47
beta cyfluthrin(I+II+III+IV)	0.1	98±2.57	91±12.80	105±9.08	0.5	94±4.76	91±8.77	107±6.12
alpha cypermethrin	0.1	103±2.22	95±5.05	102±13.62	0.5	83±2.08	107±7.75	118±2.00
Fenvalerate-(I+II)	0.1	101±1.83	89±3.72	103±10.88	0.5	98±1.81	91.51±11.89	102±8.19
Deltamethrin	0.1	105±3.18	103±6.67	90±8.06	0.5	84±3.89	88.23±8.73	111±2.55
LC-MS/MS amenable pesticides								
Carbendazim	0.01 <sup>μ</sup>	85±8.57	97±11.34	92±1.92	0.05	96±14.42	99±7.93	108±1.80
Imidacloprid	0.01	91±4.88	108±12.00	93±6.05	0.05	105±8.72	90±4.77	86±2.95
Thiacloprid	0.01	86±7.52	95±10.35	93±7.90	0.05	94±16.19	91±3.46	91±2.13
Carbofuran	0.01 <sup>o</sup>	86±6.36	109±14.22	109±5.80	0.05	94±16.20	111±5.21	97±3.93
Carbaryl	0.01	90±8.62	106±11.43	87±3.73	0.05	90±5.93	115±5.78	116±0.43
Triazophos	0.01	88±5.23	111±3.78	86±4.41	0.05	92±10.57	112±8.97	106±2.94
Monocrotophos	0.01 <sup>#</sup>	86±3.90	92±9.16	102±13.38	0.05	90±14.82	100±1.99	97±3.51
Spirotetramet	0.01	99±3.15	109±7.75	105±4.11	0.05	102±3.35	106±5.14	118±1.93
Tebuconazole	0.01 <sup>ε</sup>	97±3.50	104±5.87	97±7.74	0.05	104±7.08	110±4.39	118±0.78
Hexaconazole	0.01	110±10.13	101±9.02	115±8.59	0.05	119±1.80	109±8.31	103±5.01
Anilophos	0.01	90±4.75	102±12.08	96±3.51	0.05	88±3.79	116±2.71	111±0.55
Thiaomethoxam	0.01	93±6.89	107±10.79	101±7.52	0.05	94±1.82	98±7.58	108±3.44
Acetamiprid	0.01	89±4.83	96±5.09	104±4.52	0.05	92±5.35	85±5.22	112±2.38
Chlorfenvinphos	0.01	89±11.31	109±9.62	109±3.47	0.05	114±0.61	98±5.51	98±0.66
Propiconazole	0.01	88±8.23	94±6.13	100±12.69	0.05	85±9.20	111±3.33	99±4.33
Fenamidon	0.01	101±9.52	107±15.98	100±7.92	0.05	110±14.05	92±3.49	85±5.70
Phosphamidon	0.01	85±3.46	87±9.81	85±2.82	0.05	102±14.10	98±5.97	91±0.93
Dimethoate	0.01	98±8.85	95±8.19	95±4.91	0.05	105±15.63	99±4.42	108±0.67
Indoxacarb	0.01	97±4.39	85±7.90	90±17.73	0.05	101±3.13	86±2.78	117±3.64
Omethoate	0.01	90±8.03	104±14.53	91±10.58	0.05	92±3.01	82±2.61	88±7.12
Methomyl	0.01	107±6.93	97±13.51	94±7.50	0.05	100±4.0	89±5.40	108±5.19
Choranthraniliprole	0.01	110±3.03	108±10.74	108±9.85	0.05	108±7.02	87±6.46	95±4.39
Edifenphos	0.01	98±4.51	99±7.61	103±4.16	0.05	85±2.30	98±13.46	110±1.81
Thiodicarb	0.01	111±1.93	106±14.25	104±8.27	0.05	111±1.30	118±1.83	103±7.49
Trifloxystrobin	0.01	101±1.42	95±1.77	94±1.87	0.05	83±3.25	98±2.26	99±3.67

Table 3 (continued)

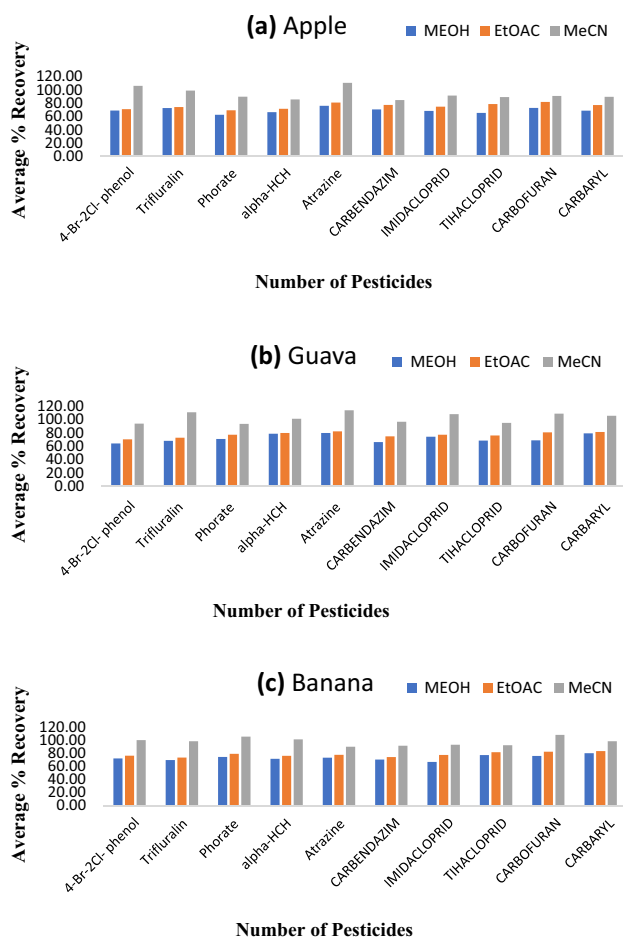
Pesticides	Fortification 10xLOQ (mg kg <sup>-1</sup> )	Average % recovery ± RSD		%ME		U (k=2) (%)				
		Apple	Guava	Banana	Apple	Guava	Banana	Apple	Guava	Banana
<i>GC-MS amenable pesticides</i>										
4-Br-2Cl- phenol	0.5	90±7.34	88±6.64	101±15.18	-12.89	15.32	-19.04	9.25	2.83	5.53
Trifluralin	0.5	101±10.80	92±2.22	89±7.13	-0.46	15.27	-19.77	15.28	5.25	2.22
Phorate	0.5	99±8.19	94±5.82	87±5.05	15.31	19.74	-18.24	18.74	9.28	9.26
alpha-HCH	0.1	84±4.56	103±10.29	100±2.29	1.50	-19.90	-21.32	15.26	2.26	11.28
Dimethoate	0.5	103±2.31	98±7.25	107±4.29	5.16	-14.52	4.23	17.28	8.55	12.03
Atrazine	0.5	108±6.45	108±6.88	91±6.71	-18.65	-16.63	10.59	8.25	10.25	17.12
beta-HCH	0.1	92±9.57	96±4.11	109±7.28	20.77	-20.10	-17.92	9.78	8.14	19.52
Lindane	0.1	96±10.64	91±7.10	112±3.86	1.90	-16.83	-13.25	4.29	2.29	20.25
Phosphamidon	0.5	95±5.13	106±2.23	87±7.12	-17.00	10.27	-7.24	14.88	6.22	16.46
Chlorothalonil	0.1	91±4.21	98±7.08	101±4.06	22.65	-20.59	-14.71	10.29	9.88	4.56
delta-HCH	0.1	99±7.17	85±6.53	93±7.00	-12.05	-18.41	-20.70	13.50	5.27	7.89
Dimethachlor	0.5	91±3.29	114±4.90	103±3.25	5.30	8.51	4.34	18.75	6.99	8.90
Alachlor	0.5	91±1.25	108±2.59	111±6.32	21.95	19.45	-19.07	23.54	2.17	2.55
Parathion-methyl	0.5	91±4.41	90±5.10	98±1.18	19.91	19.37	12.60	11.48	2.23	1.55
Heptachlor	0.5	99±5.77	105±4.85	107±3.93	-18.75	-20.21	-4.95	22.80	9.87	4.22
Malathion	0.5	95±4.52	110±13.25	116±2.02	0.69	19.27	13.82	15.87	8.59	2.44
Chlorpyrifos	0.5	103±8.27	87±9.15	107±6.56	-16.59	5.36	2.50	25.84	4.58	2.99
Aldrin	0.5	109±5.31	96±17.31	106±1.79	-13.44	-18.67	20.00	7.29	18.20	14.84
Pendimethylene	0.5	98±6.62	109±4.18	90±3.88	17.65	18.47	-5.37	8.41	3.18	4.02
Quinalphos	0.5	108±6.97	90±6.28	83±2.02	-17.90	-12.75	7.64	10.20	8.25	9.99
op-DDD	0.1	102±9.31	98±4.61	105±8.55	-15.79	14.70	-18.64	19.50	11.47	8.27
Butachlor	0.5	110±8.87	116±9.68	82±1.50	21.47	7.85	-19.90	17.47	19.25	11.12
alpha-endosulfan	0.5	90±10.27	97±7.58	103±5.32	-20.17	-16.65	-16.73	19.50	21.58	18.29
pp-DDD	0.1	94±8.91	104±5.23	111±6.22	10.23	12.23	-8.46	7.88	10.56	4.25
Profenophos	0.5	87±5.58	94±11.76	108±7.32	-14.63	1.95	-20.85	23.22	6.20	9.26
pp-DDE	0.1	81±4.973	86±5.91	108±6.50	-11.46	-20.35	-18.55	9.53	6.22	7.14
op-DDT	0.1	102±3.18	107±11.14	93±9.80	-17.42	-18.53	-1.75	17.43	5.62	2.63
beta-endosulfan	0.1	94±8.91	112±6.46	94±7.24	13.33	-14.28	13.71	17.84	3.22	1.58
Ethion	0.5	111±5.20	111±6.28	113±3.14	-18.82	-17.78	-16.22	12.45	5.55	6.27
pp-DDT	0.1	108±5.32	104±10.26	87±3.00	3.53	-15.36	-33.99	18.44	2.23	9.25
Endosulfan sulphate	0.1	96±11.06	109±12.51	106±10.28	-16.54	6.46	16.25	16.11	6.58	4.25
Spiromesifen	1.0	103±4.06	106±5.89	111±1.70	18.81	17.47	-19.36	4.28	9.25	6.80
Bifenthrin	1.0	104±9.73	90±8.20	98±2.77	-20.42	-19.91	18.35	8.47	18.52	22.25



Table 3 (continued)

Pesticides	Fortification 10xLOQ (mg kg <sup>-1</sup> )	Average % recovery ± RSD			%ME			U (k=2) (%)		
		Apple	Guava	Banana	Apple	Banana	Guava	Apple	Banana	Guava
Fenpropathrin	1.0	96±3.06	88±7.36	112±0.59	-1.25	6.86	-3.46	9.14	14.44	7.52
lamda cyhalothrin	1.0	102±6.96	95±9.80	81±2.68	19.69	2.44	20.26	15.88	19.22	9.60
beta cyfluthrin(I+II+III+IV)	1.0	99±5.33	90±5.48	114±3.14	-18.07	-0.58	19.59	18.46	13.20	19.65
alpha cypermethrin	1.0	98±12.19	100±9.26	104±7.34	-7.22	-0.38	-18.68	12.80	7.52	5.26
Fenvalerate-(I+II)	1.0	93±5.92	86±4.18	116±3.05	21.91	-12.94	-14.17	10.55	9.88	2.03
Deltamethrin	1.0	85±5.09	111±7.90	104±1.07	7.37	-10.20	-16.44	26.85	2.33	5.69
<i>LC-MS/MS amenable pesticides</i>										
Carbendazim	0.1	82±2.90	84±3.94	94±3.20	-11.26	-9.07	18.92	5.87	11.23	9.20
Imidacloprid	0.1	85±6.08	112±8.47	90±10.03	-13.77	-10.55	-17.85	2.26	5.60	3.84
Thiacloprid	0.1	96±11.18	100±9.70	82±3.87	-9.56	9.82	-17.96	16.20	9.20	8.22
Carbofuran	0.1	83±6.80	112±6.19	87±7.45	-8.96	25.69	4.28	2.20	7.14	5.82
Carbaryl	0.1	88±10.21	118±2.10	88±4.62	1.84	-17.90	13.82	8.44	12.55	3.26
Triazophos	0.1	91±14.97	118±4.10	89±12.20	10.96	-20.80	-6.31	17.22	5.45	8.28
Monocrotophos	0.1	80±10.71	90±7.75	95±12.79	-5.18	-16.09	5.91	16.33	10.22	9.25
Spirotetramet	0.1	89±7.66	111±5.30	93±15.61	-5.38	3.73	-17.04	13.20	3.22	5.60
Tebuconazole	0.1	90±1.00	118±1.93	89±4.95	7.27	3.93	13.94	4.59	2.20	5.88
Hexaconazole	0.1	92±8.59	107±5.54	98±17.75	14.19	-14.29	-19.01	5.55	9.85	2.22
Anilophos	0.1	82±3.26	115±5.83	87±8.77	-20.34	14.07	13.64	6.90	5.45	2.54
Thiomethoxam	0.1	106±2.58	117±1.98	87±4.24	5.55	-19.35	8.00	7.15	11.27	19.55
Acetamiprid	0.1	81±3.81	93±11.70	88±5.20	-8.70	16.58	-3.38	4.87	18.50	10.55
Chlorfenvinphos	0.1	101±13.76	116±3.65	94±12.96	-19.26	19.11	-6.71	2.22	6.57	9.80
Propiconazole	0.1	93±17.79	95±4.31	82±8.80	-0.14	18.59	-9.91	5.54	6.23	15.20
Fenamidon	0.1	83±16.48	108±8.39	79±8.21	6.96	-20.25	-12.24	17.45	20.12	19.52
Phosphamidon	0.1	81±0.24	88±6.11	87±6.44	-19.95	-12.71	13.60	13.22	7.80	4.52
Dimethoate	0.1	84±8.91	103±10.65	87±4.02	-16.32	-19.59	18.84	2.79	5.56	2.20
Indoxacarb	0.1	100±15.32	99±7.94	90±17.73	8.30	2.50	-11.67	11.45	19.52	14.27
Omethoate	0.1	86±1.45	89±13.38	84±6.16	-17.40	-20.24	17.76	17.45	10.25	8.44
Methomyl	0.1	97.4±6.0	94±13.54	86±2.39	18.90	15.23	12.28	5.94	7.52	5.56
Choranthraniliprole	0.1	99±3.17	107±7.51	83±8.42	10.12	11.63	-5.03	18.45	14.22	5.54
Edifenphos	0.1	95±13.07	112±2.29	95±6.66	-9.03	-20.65	18.81	10.32	17.45	12.52
Thiodicarb	0.1	117±2.6	110±11.75	114±18.33	14.80	7.23	-19.35	11.28	16.25	19.89
Trifloxystrobin	0.1	91±10.92	97±1.82	86±2.74	-19.76	20.33	23.37	12.23	10.55	12.45

MRL<sup>a</sup> 0.05 mg kg<sup>-1</sup>, <sup>b</sup>1.0 mg kg<sup>-1</sup>, +2.0 mg kg<sup>-1</sup>, <sup>c</sup>0.2 mg kg<sup>-1</sup>, <sup>d</sup>4.0 mg kg<sup>-1</sup>, <sup>e</sup>0.5 mg kg<sup>-1</sup>, <sup>f</sup>0.1 mg kg<sup>-1</sup>, <sup>g</sup>9.5 mg kg<sup>-1</sup>, <sup>h</sup>1.0 (apple) 1.5 (banana) mg kg<sup>-1</sup>, <sup>i</sup>5.0 (apple and guava), 1.0 (banana) mg kg<sup>-1</sup>



**Fig. 1** Comparison of average % recovery of 64 pesticides using MeOH, EtOAc and MeCN as extracting solvents in case of **a** apple, **b** guava and **c** banana matrices fortified at LOQ level

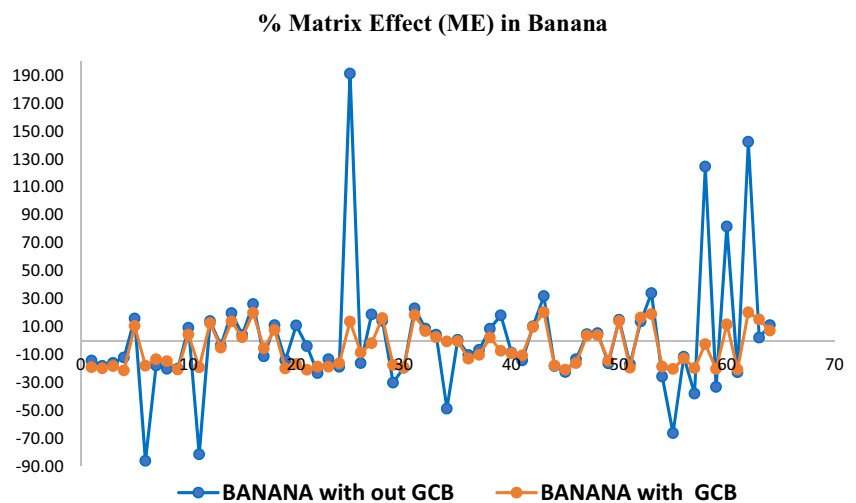
because it gave the highest average percent recovery in all three matrices and for all the pesticides. The research works of [19–21] resulted the use of acetonitrile as good extracting solvent. Present study used PSA to absorb matrix co-extractives,  $MgSO_4$  to remove water and combination of PSA + GCB only for banana to remove heteropolysaccharide and carotenoids which are otherwise not removed by PSA alone. Okihashi and his associates [22] reported the role of GCB in case of banana extract. Saito and his co-workers [23] also reported that the combination of GCB + PSA provided excellent clean-up for removal of matrix materials. In our study, using GCB as cleaning up agent for banana matrices showed promising result. Without using GCB, beta-endosulfan (191.49%), indoxacarb (124.77%), trifloxystrobin (142.59%) and Choranthraniliprole (81.81%) suffered very high matrix enhancement effect. GCB reduced these values to 13.71%, – 2.50%, 20.33% and 11.63% respectively. Some pesticides also suffered strong matrix suppression effects viz beta-HCH (– 86.11%),

alachlor (– 81.40%), pp-DDT (– 30.09%) and beta-cyfluthrin (– 48.74%) which were reduced to – 17.92%, – 19.07%, – 17.39% and – 0.58% respectively. Comparison of matrix effect in presence and absence of GCB in banana matrix was depicted in Fig. 2.

### 3.2 Efficiency of the method

A total of 39 pesticides were analysed in GC–MS. On the other hand, 25 pesticides were analysed in LC–MS/MS. In GC–MS analysis, at LOQ level, 86–111%, 87–110%, 84–114% recovery was found respectively for apple, banana, guava with respective relative standard deviation (RSD) ranging from 0.47 to 13.45%, 3.89 to 16.88%, 2.23 to 12.80%. Irrespective of the pesticides and matrices, pp-DDT acquired the least recovery of 84.44% with RSD value 5.43%. In GC–MS analysis, instrument parameters are standardized to increase the sensitivity and selectivity of the instrument. Considering the GC injection mode, split mode has been chosen to avoid the overloaded peaks that reduced the separation efficiency of the column. The GC oven temperature programming was standardized so that the analyte is well separated having good peak shape and the matrix interference is minimized to increase the sensitivity. Four different other methods were compared with the present method regarding the standardization of GC–MS. The present method took 50 min run time in comparison to 70.33 min [16], 60.17 min [24], 60 min [25], 55 min [26]. The less run time of the present method in comparison to others was established. Standardization of MS parameters was done by identification of peaks in total ion chromatogram of mixed standard solution in scan mode by their specific RT and characteristic mass fragmentation pattern. The most abundant ion that had the highest S/N ratio and showed no matrix interference was selected as quantifier ion. The other two ions were selected as qualifier. A SIM method is prepared by fixing the RT window of each compound from the full scan chromatogram and the RT of individual compounds. Twenty-five pesticides, analysed in LC–MS/MS for apple, banana and guava matrices resulted the recovery range of 85–111%, 84–114% and 86–110% at LOQ level respectively with the respective RSD values of 1.42–11.31%, 1.87–13.38% and 1.77–14.53%. LC–MS/MS conditions have been standardized to achieve good separation, satisfactory selection and increased sensitivity which enable to analyse samples having complex matrices with a high degree of confidence. Different combination of mobile phases were tested because mobile phase has the direct effect on the peak shape and the retention time of the analyte in the column as well as on MS sensitivity. The different combination of water, methanol, acetonitrile with ammonium acetate, ammonium formate buffers were surveyed.

**Fig. 2** Comparison of %ME of 64 pesticides while cleaning up with and without GCB in Banana matrix



The well defined shape and reproducibility of retention time of pesticides were achieved by using mobile phase (A) water with 5 mM ammonium formate (B) methanol with 5 mM ammonium formate by using reverse phase Zorbax SB-C18 (4.6 × 150 mm, 5 μm) column. The total ion chromatograms (TIC) of 0.1 μg ml<sup>-1</sup> for GC-MS and LC-MS/MS are presented in Fig. 3.

### 3.3 Method validation

The LOD and LOQ of the GC amenable pesticides were found to be within the ranges of 0.001–0.04 mg kg<sup>-1</sup> and 0.005–0.11 mg kg<sup>-1</sup> respectively. The linearity of the calibration curve was established with R<sup>2</sup> value in the range of 0.988–0.999. In case of LC amenable pesticides, the values for LOD, LOQ, R<sup>2</sup> were within the range of 0.001–0.008 mg kg<sup>-1</sup>, 0.008–0.01 mg kg<sup>-1</sup> and 0.971–0.999 respectively. The mean recoveries were found in the range of 80–120% (Table 3). Good accuracy was observed for all analytes with relative standard deviation ≤ 20% which is as per the requirements of SANTE regulating the performance of analytical method. Relative percentage deviation (%RPD) was calculated for inter and intraday assay recovery for three matrixes at LOQ level. The recovery precision was expressed by average recovery percentage ± SD along with RSD value. The inter- and intra-day precision of the method for apple matrix were found to be respectively < 13% and < 17% and %RPD value was < 12%. For guava matrix respective inter and intraday values were < 16% and < 17% and %RPD value was < 10% and inter and intraday values for banana were < 19% and < 16% respectively and %RPD value was < 18%. All these values of precision satisfy SANTE and European Commission guidelines (Fig. 4) and therefore the method is precise. To define the quality of analytical results, both traceability and degree of confidence are equally important. The

uncertainty was determined at the LOQ level for all the pesticides as per the EURACHEM/CITAC (Table 3) showed MU values for individual pesticides with the majority of compounds having uncertainties < 20%. In apple, %ME values ranged between (– 20.42 and 22.65%) and therefore are almost within ≤ 20%. The exceptions are chlorothalonil (22.65%), alachlor (21.95%), butachlor (21.75%) and fenvalerate (21.91%) which were undergonematrix enhancement effect. Whereas in guava, only one pesticide trifloxystrobin was found to have matrix enhancement effect (23.37%). All the % ME values were presented in Table 3 for GC-MS and LC-MS/MS respectively. In case of banana, 15% pesticides showed matrix suppression effect out of 64 pesticides, whereas matrix enhancement effect was found for 12% pesticides. A comparison for banana matrix with presence and absence of GCB as cleaning up agent was designed for showing the matrix effect in Fig. 2.

### 3.4 Analysis of real samples

The method was successfully applied to analyse of market samples of apple, banana, guava which were collected from four different districts (Kolkata, Howrah, Hooghly and Burdwan) of West Bengal, India and detected different numbers of pesticides (Table 4). Among the samples, guava collected from Pandua and Howrah were detected with chlorpyrifos (0.25 ± 6.19) and profenophos (0.62 ± 1.61) respectively. Two pesticides namely carbendazim (0.9 ± 2.32) and quinalphos (0.12 ± 1.26) were detected in banana samples collected from Howrah. Apple sample collected from Pandua was detected with dimethoate (0.18 ± 4.20). But apple sample collected from Kolkata was detected with three pesticides namely trifloxystrobin (0.05 ± 5.88), tebuconazole (0.10 ± 6.37) and carbendazim (0.05 ± 4.37). Therefore, highest numbers of pesticides

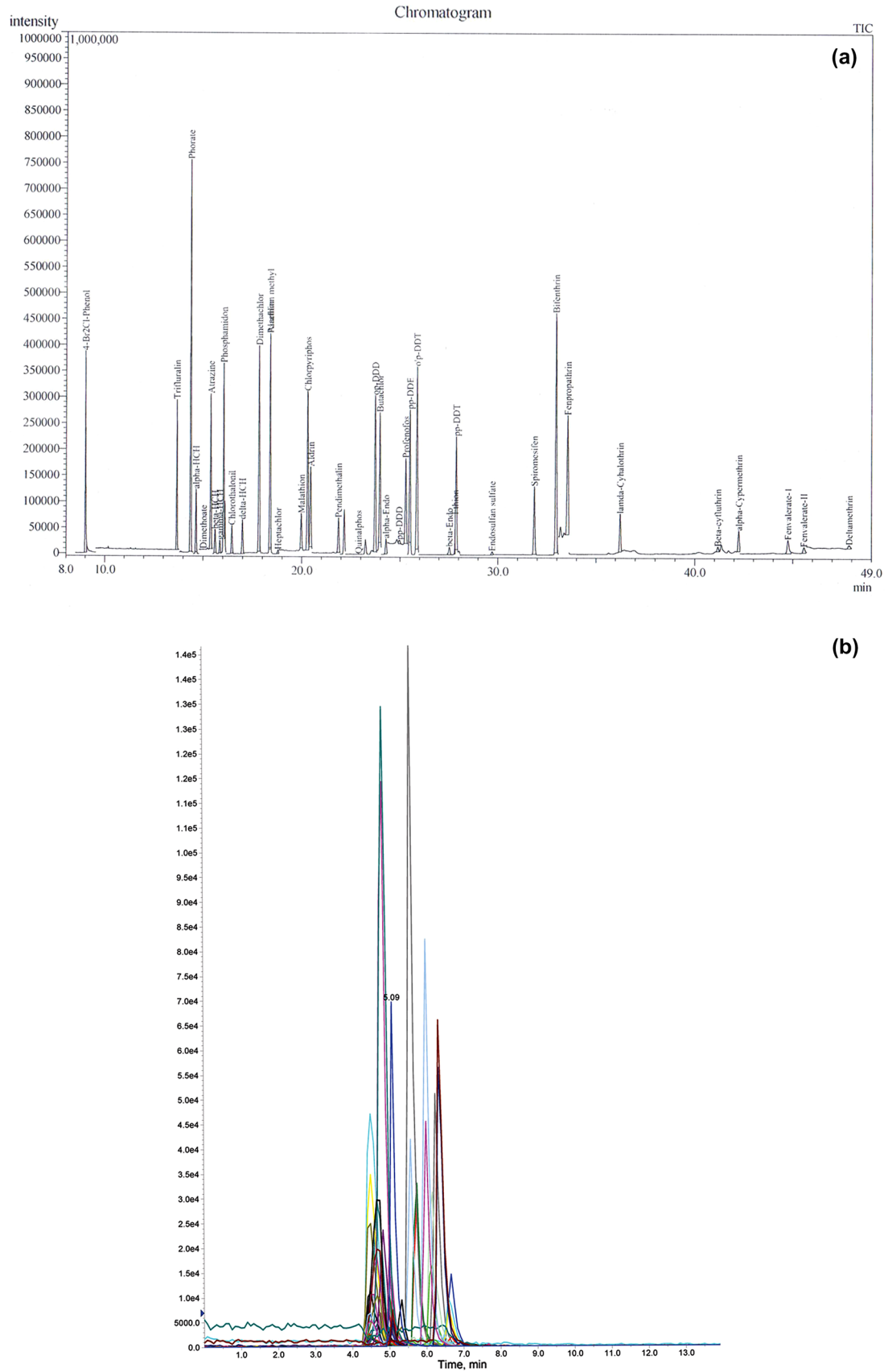
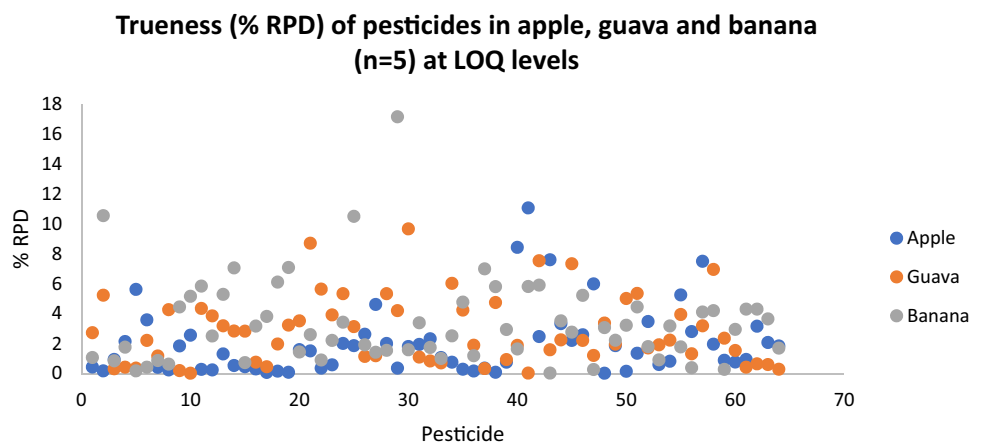


Fig. 3 TIC of 0.1 ugml-1 of **a** GC-MS and **b** LC-MS/MS

**Fig. 4** Comparison of Inter and Intra- assay precision recovery of pesticide residues in apple, guava and banana matrixes



**Table 4** Analysis of market samples of apple, banana and guava

Location	Substrate	Number of samples		Pesticides detected ( $\mu\text{g g}^{-1} \pm \text{RSD}$ )	Mode of analysis (GC-MS/LC-MS/MS)
		Analysed	Detected		
Pandua	Apple	12	01	Dimethoate ( $0.18 \pm 4.20$ )	GC-MS
Kolkata	Apple	12	01	Trifloxystrobin ( $0.05 \pm 5.88$ ) Tebuconazole ( $0.1 \pm 6.37$ ) Carbendazim ( $0.05 \pm 4.37$ )	LC-MS/MS
Pandua	Guava	12	01	Chlorpyrifos ( $0.25 \pm 6.19$ )	GC-MS
Hawrah	Guava	12	01	Profenophos ( $0.62 \pm 1.61$ )	LC-MS/MS
Hawrah	Banana	12	02 (01 + 01)	Carbendazim ( $0.09 \pm 2.32$ ) Quinalphos ( $0.12 \pm 1.26$ )	LC-MS/MS

(3) were detected in the apple samples collected from Kolkata.

## 4 Conclusion

In this era of good health and diet consciousness, food stuffs are being monitored regularly to check the presence of pesticide residues. A quick, accurate, precise and efficient method is therefore necessary to detect and determine pesticide residues in real samples. The present method is validated as per Eurachem [14] and SANTE guidelines [15]. The efficiencies of methanol, ethyl acetate and acetonitrile were checked as extracting solvents and finally acetonitrile was chosen as extracting solvent in the method. The use of GCB + PSA mixture as cleaning up agent in case of banana reduced the interference of heteropolysaccharide and carotenoids and thus nullified matrix interferences. The present method can be used for both the instruments at a time. This modified QuEChERS method is useful for quick determination of multiclass

multipesticide residues in apple, guava and banana meant for export.

**Funding** The authors are grateful to Indian Council of Agricultural Research (ICAR), New Delhi, India for the financial support.

## Compliance with ethical standards

**Conflict of interest** We all authors declare that we have no conflict of interest.

**Human and animal rights** This article does not contain any study with human participants or animals performed by any of the authors.

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