Validation of Multi Residue Method with Mixture of Multi-Class Pesticides in Bitter Gourd by Gas Chromatography Coupled with Triple Quadrupole Mass Spectrometry

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A new multi-residue method has been developed and validated for the routine analysis of 64 multi-class pesticide residues in bitter gourd (*Momordica charantia* L.) by gas chromatography coupled with triple quadrupole mass spectrometry in a single run of 49.67 min. The method was successfully applied for the analysis 10 varieties of cucurbits (ash gourd, bitter gourd, little gourd, cucumber, ridge gourd, bottle gourd, snake gourd, water melon, gherkin and pumpkin). Samples were extracted by the application of a single phase extraction of 15 g of sample with 30 mL of acetonitrile, followed by a liquid-liquid partition formed by the addition of 1.2 g of $MgSO_4$. A dispersive solid-phase extraction with 0.4 g of primary secondary amine (PSA) was applied to clean up the extract. The method was properly validated and the values of some merit figures, such as recovery, precision, linearity, detection limit and quantification limit for each pesticide were calculated. The average recoveries in bitter gourd obtained for each pesticide ranged between 70-130 % at three fortification levels (0.01, 0.05 and 0.1 mg kg^{-1}). The percentage of relative standard deviation (% RSD) is lower than the 20 %. The established limit of detection (LOD) is 0.001 mg kg^{-1} and limit of quantification were (LOQ) 0.01 mg kg^{-1}.

Keywords: QuEChERS extraction, Triple quadrupole mass spectrometry, Multiresidue analysis, Bitter gourd.

INTRODUCTION

The application of wide variety of phytosanitary products in vegetables is a usual practice in modern agriculture in order to increase production yield and obtain high quality products. However these pesticides can remain in vegetables as residues. The toxicity of these compounds got importance in the monitoring of vegetable quality in order to avoid the risks to the consumers, as well as to regulate international trade. The government bodies of different countries and different international organizations have established the maximum residue limits (MRLs) for each compound and commodity to ensure the food safety.

This has led to got importance in the development of multi-residue analysis methods, which allows the detection and quantification of multi class pesticide residues in a single run at very low concentration which is below the maximum residue limits established by the legislations of many countries[1-4]. In order to achieve this, the samples throughput has to be increased, by reducing the analytical analysis time and preparation of sample has to meet the international standards in pesticide residue analysis[5,6]. Many of previous extraction cleanup techniques fail in performance in multiresidue applications[7]. Rapid, simple and robust extraction methods are required in routine analysis of pesticide residue laboratories. The QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction and cleanup procedure has been following in many residue laboratories because of obtaining high recovery values even in very low concentrations, simple instrumentation and materials and low cost per sample. This method is based on acetonitrile extraction and induced partition by addition of $MgSO_4$ and NaCl. The dispersive solid-phase extraction with primary secondary amine (PSA) is applied as clean up[8,9].

The pesticide residue laboratories are looking for development of new methods in the analysis of pesticide residues on gas chromatography coupled with mass spectrometry based methods, these methods mainly focused on accuracy, repeatability and reproducibility of result and moreover on reducing

the analysis time. The triple quadrupole mass analysers (QqQ) are powerful detectors with an enhanced selectivity and data acquisition speed these will allows the simultaneous monitoring of a high number of coeluting compounds. The QqQ is able to operate in different modes, such as full scan, multi reaction monitoring (MRM) and selected ion monitoring (SIM). The full scan and multi reaction monitoring modes are essential acquisition modes to simultaneously identification, quantification and confirmation of pesticide residues in trace analysis of complex matrices.

The pesticide residue analysis is not only necessary to obtain the results in a short time but also the results must be obtained within a quality system[7]. The current work was properly validated under the guidelines of European SANCO and the ISO 17025 norms.

The aim of this study is the development and validation of a multi residue method suitable to determine about 64 multi-class pesticides in bitter gourd. The extraction and clean-up steps were based on the QuEChERS method. The target compounds were finally determined in 49.67 min.

EXPERIMENTAL

Analytical grade standards were procured from Dr. Ehrenstorfer and Sigma Aldrich (Germany), activated anhydrous magnesium sulphate GR grade, activated anhydrous sodium sulphate GR grade, primary secondary amine (PSA) sorbent (Agilent Technologies) acetonitrile, *n*-hexane, acetone sodium chloride all from Merck were purchased from local distributor. Stock standard solutions of 500 µg mL^{-1} each compound was prepared by weighing of powder or liquid and dissolved in 9:1 hexane:acetone mixture in 25 mL A grade glass volumetric flask, then the solutions was stored in refrigerator at -20 °C. A multicompound mixture of working standard solution 1 µg mL^{-1} was prepared by adequate dilution of the corresponding stock solution with *n*-hexane and stored under refrigerator at -20 °C.

Extraction and clean-up procedure: Bitter gourd samples were purchased from local vendor, a portion of 1 kg sample was chopped with robot coupe blixer. Weighed out of 15 ± 0.1 g sample in to 50 mL centrifuge tube, added 30 ± 0.1 mL acetonitrile to the 50 mL centrifuge tube cap well and shacked for 1 min, homogenized the sample at 14000-15000 rpm for 2-3 min using Heidolph silent crusher, added 3 ± 0.1 g sodium chloride and mixed it by shaking gently then centrifuged for 3 min at 2500-3000 rpm to separate the organic layer, taken approximately 16mL of organic layer to the test tube and add 9 ± 0.1 g anhydrous sodium sulphate to remove the moisture content.

Dispersive solid phase cleanup (d-SPE-Cleanup): Weighed out 0.4 ± 0.01 g primary secondary amine sorbent and 1.2 ± 0.01 g anhydrous magnesium sulphate in to 15 mL centrifuge tube for 8 mL organic layer (extract), transferred 8 mL extract in to 15 mL centrifuge tube with primary secondary amine and anhydrous magnesium sulphate cap the tube well and vortex for 30 sec then centrifuged the tube for 5 min at 2500-3000 rpm, transferred 2 mL extract to the test tube and evaporate the solvent (acetonitrile) using turbovap concentrator, reconstitute with 1 mL *n*-hexane for GC- MS/MS analysis.

The matrix-matched calibration standards were prepared with blank sample, a blank sample was extracted applying the same procedure and the corresponding volumes of multi-compound working standard solution were added in the centrifuge tube before the clean-up stage.

GC-MS/MS analysis: Samples were analyzed on GC-MS/MS, BRUKERS SCION 436- GC TQ equipped with triple quarter pole mass spectrometer and connected with Zebron 5 MS column (30 m × 0.25 mm id) coated with 5 % phenylmethyl poly siloxane with 0.25 µm film thickness. The general operating conditions were as follows: the column oven temperature programme: initially 50 °C for 3 min holds, increase @ 20 °C/min to 150 °C, hold for 0.00 min, @ 3 °C/min to 230 °C, hold for 5 min, @ 10 °C/min to 290 °C hold for 4 min the total run time was 49.67 min. Injector port temperature: 260 °C. Constant column flow 1 mL/min. Carrier gas: helium, Injection volume: 1 µL. Split ratio 1:10. Electron energy: 70 eV. Filament current: 80 µA. EI filament used: 2. Collision cell pressure: 1. 535 m torr. Manifold temperature: 40 °C. Ion source temperature: 219.8 °C. Transfer line temperature: 249.1 °C.

For linearity range, the calibration samples were prepared with stock solutions in blank bitter gourd extract. Calibration ranges are kept between 0.01 to 0.125 mg kg^{-1}. The relative standard deviations (RSD) for individual points of calibration curve were calculated. The fortification and recoveries were assessed using spiked blank samples at three concentration levels. The three levels of spiked samples were prepared by adding the adequate amount of working standard mix and each concentration was replicated in to trice. All the replicated samples were allowed for run and calculated the R.S.D. and recovery values for each. The limit of detection (LOD) and limit of quantification (LOQ) were determined by signal-to-noise (S/N) ratios of 3 and 10 respectively.

RESULTS AND DISCUSSION

The MS parameters are developed for 64 compounds in the mixture. First, the compound was monitored in full scan mode in mass by charge *(m/z)* range from 50- 400. Identified the retention times of each compound in this run. Then, the precursor ion was selected for collision-induced dissociation (CID) basing on the highest *m/z* ration. The collision-induced dissociation is performed with argon gas at ranging from 10 eV to 70 eV. A minimum of two MS/MS transitions was selected for each compound. The triple quadrupole mass analyzer always works in electron ionization mode as the suitable technique for the majority of compounds. Finally, the MS/MS parameters were for 64 compounds were optimized in full scan mode.

The multi reaction monitoring mode applied in QqQ analyzers provides a high increase in selectivity since the analyzer is focused on selected in first (Q1) and third quadrupoles (Q3). In consequence, a sensitivity gain results from the greater signal-to-noise ratio measured, in spite that the ion transmission diminishes due to path length between the ion source and the electron multiplayer[7]. The multi reaction monitoring transition was performed simultaneously and identified the qualifier and quantifier ions for all the compounds in the standard mixture (Table-1).The QqQ analyzer is having the high scan speed it

| | | | | TABLE-1 | |
| | | | | GC-MS/MS CONDITIONS | |
Compound	Retention time	Molecular weight	Monitoring Ions	Precursor Ion	Qualifier Ion	Quantifier Ion
Methamidophos	8.54	141.34	141, 94	141	141 > 64, 141 > 79, 141 > 95	141 > 95
Dichlorvos	8.62	220.98	237, 235	185	185 > 63, 185 > 93, 185 > 109	185 > 93
Monocrotophos	15.45	223	192, 127, 164	127	127 > 109, 127 > 95, 127 > 79	127 > 109
Phorate	15.71	276	260, 231, 121	260	260 > 175, 260 > 231, 121 > 93	121 > 93
Alpha HCH	15.84	290.82	219, 181, 183	219, 181	219 > 183, 219 > 147, 181 > 145	181 > 145
Dimethoate	16.45	229.28	125, 229, 93, 87	125, 229	125 > 79, 125 > 93, 125 > 125, 125 > 87	125 > 125
Beta HCH	17.00	290.82	219, 181, 183	181, 219	181 > 145, 219 > 183	181 > 145
Atrazine	17.09	215.68	215, 200	215	215 > 200, 215 > 172, 215 > 138	215 > 200
Lindane	17.36	290.8	181, 219, 183	181, 219	181 > 145, 219 > 183	181 > 145
Chlorthalanil	18.14	265.91	266	266	266 > 133, 266 > 168, 266 > 231	266 > 231
Diazinon	18.15	304.3	304, 779, 179	304, 179	304 > 137, 304 > 164, 304 > 179, 179 > 137	179 > 137, 304 > 137
Delta HCH	18.80	290.82	219,183, 181	181, 219	181 > 145, 219 > 183	181 > 145
Phophomidon	20.04	299	264, 127	264	264 > 72, 264 > 127, 264 > 193	264 > 127
Chlorpyrifos methyl	20.35	322.53	286, 125	286	286 > 208, 286 > 241	286 > 241
Methyl parathion	20.71	263.21	263, 223, 125	263	263 > 109, 263 > 127, 263 > 246	263 > 109
Alachlor	20.81	269.76	188, 369, 238, 240	188, 269	188 > 160, 188 > 130, 269 > 160, 269 > 188	188 > 160, 269 > 160
Heptachlor	20.97	373.32	337, 274, 272	272	272 > 237, 272 > 141, 272 > 117	272 > 237
Metalaxyl	21.25	279	206	206	206 > 132, 206 > 162, 206 > 206	206 > 206
Demeton-S-methyl sulfone	21.70	290.34	142, 109, 169	169	169 > 109, 169 > 125	169 > 125
Fenitrothion	22.11	277	277, 260	260,277	260 > 109, 260 > 125, 260 > 151, 277 > 109, 277 > 260	260 > 109, 277 > 109
Malathion	22.79	330.36	173, 127, 125	173	173 > 99, 173 > 117, 173 > 127	173 > 99
Aldrin	22.83	364.91	263,286, 314, 293	263	263 > 193, 263 > 228	263 > 193
Chlorpyrifos	22.99	350.62	314, 286, 197	314,286	314 > 166, 314 > 258, 314 > 286, 286 > 93, 286 > 271	314 > 258
Fenthion	23.24	278	278, 169	278	278 > 109, 278 > 125, 278 > 245	278 > 109
Parathion	23.43	291.3	291, 261, 235	291	291 > 109, 291 > 137	291 > 109
Dicofol	23.71	370.48	250, 251, 759	251	251 > 139, 251 > 111	251 > 139
Dieldrin	23.71	380.9	277,263	277,263	277 > 241,277 > 206,277 > 170,263 > 193, 263 > 228	263 > 193
Fipronil	25.27	437.15	367, 369, 351, 213	367	367 > 178, 367 > 213, 367 > 255	367 > 213
Chlorfenvinphos	25.51	359.57	323, 267	267, 323	267 > 159, 323 > 267	323 > 267
Quinolphos	25.76	298	298, 146, 157, 118	298, 146, 157	298 > 129, 298 > 156, 298 > 190, 146 > 118, 157 > 129	146 > 118
Allethrin-a	26.00	302.41	125,135,169,107	125	125 > 81, 123 > 95	125 > 81
Allethrin-b	26.00	346.42	125,135,169,107	125	125 > 81, 123 > 95	125 > 81
2,4 DDE	26.70	318.03	237, 235	246, 318, 163, 226	246 > 176,318 > 318,318 > 246, 163 > 127, 226 > 206	246 > 176
Alpha endosulfan	27.05	406.93	241, 265, 277, 243	241, 265	241 > 206, 241 > 170, 265 > 229, 265 > 195, 265 > 193	241 > 206
Butachlor	27.21	311.9	237, 323, 240, 266	237, 323	237 > 160, 237 > 188, 176 > 134, 176 > 146, 188 > 130	176 > 146
Hexaconazole	28.01	314.21	214, 175	214	214 > 124, 214 > 152, 214 > 172	214 > 172
Fenamiphos	28.47	303.3	303,288, 154	303	303 > 139, 303 > 154, 303 > 180	303 > 154
Profenophos	28.47	372	339, 139, 559, 759	339, 139	339 > 188, 339 > 251, 339 > 269, 139 > 97	139 > 97
4,4-DDE	28.61	318.03	318, 246	318, 246	318 > 176, 318 > 246, 246 > 176, 318 > 318	318 > 318
2,4-DDD	28.91	320.05	237, 235	235	235 > 165, 235 > 200, 235 > 139	235 > 165
Endrin	29.72	380.93	281, 263, 317, 245	281, 263	281 > 173, 281 > 209, 281 > 245, 263 > 193, 263 > 228	263 > 193
Beta endosulfan	30.42	406.93	241, 195	195, 241	195 > 159, 241 > 206	195 > 159
4,4-DDD	31.02	320.05	237, 235	235	235 > 165, 235 > 199, 235 > 200	235 > 165
2,4-DDT	31.02	354.49	237, 235	235, 141	235 > 200, 235 > 235, 141 > 95	141 > 95
Ethion	31.25	384.48	231, 384, 257, 153	231	231 > 129, 231 > 175, 231 > 203	231 > 129
Triazophos	32.15	313	257, 161	257	257 > 119, 257 > 134, 257 > 162	257 > 162
Endosulfan sulphate	32.67	422.92	274, 272, 387	272, 387	272 > 141, 272 > 165, 272 > 237, 387 > 253	272 > 237

Compound	Retention time	Molecular weight	Monitoring Ions	Precursor Ion	Qualifier Ion	Quantifier Ion
4,4-DDT	33.18	354.49	237, 235	235	235 > 165, 235 > 199, 235 > 200, 235 > 235, 235 > 199	235 > 165
Trifloxystrobin	33.33	408.37	222, 116, 190	222, 116, 190	222 > 190, 222 > 162, 222 > 130, 116 > 89, 190 > 130	116 > 89
Tebuconazole	34.20	307.8	250, 125	250	250 > 125, 250 > 153, 250 > 163	250 > 125
Bifenithrin	36.71	422.87	181, 165, 166	181, 165	181 > 115, 181 > 165, 181 > 166, 165 > 115	181 > 166
Methoxychlor	36.83	345.7	228, 227	227	227 > 169, 227 > 184	227 > 169
Fenpropathrin	37.30	349	265, 165, 181,125	265,165,181	265 > 210, 265 > 181, 165 > 153, 181 > 152	181 > 152
Phosalone	38.66	367	367, 182	367, 182	367 > 111, 367 > 138, 367 > 182, 182 > 138, 182 > 111	367 > 111, 182 > 111
Lambda cyhalothrin	40.97	449.9	181, 797	181, 797	181 > 127, 181 > 152	181 > 152
Azinphos ethyl	41.28	345.4	160, 134, 155, 127	160,134,155, 127	160 > 102, 160 > 105, 160 > 132	160 > 132
Permethrin-I	42.9	390	183, 163	163, 183	163 > 127, 183 > 153	183 > 153
Permetrin-II	43.21	390	183, 163	163, 183	163 > 127, 183 > 153	163 > 127
Cyfluthrin	44.48	434.3	226, 206, 163	206, 163, 226	206 > 151,206 > 177,206 > 179, 163 > 127, 226 > 206	206 > 177
Cypermethrin	44.64	416.32	163, 181, 165, 127	163, 181	163 > 127, 181 > 152	163 > 127
α-Cypermethrin	44.92	406.93	241, 265, 277, 243	241, 265	241 > 206,241 > 170,265 > 229, 265 > 195, 265 > 193	241 > 206
Fenvalarate	46.04	419	225, 167	225	225 > 91, 225 > 119, 225 > 147	225 > 119
Fluvalinate-I	46.30	502.93	250, 199, 157	250	250 > 55, 250 > 200	250 > 200
Fluvalinate-II	46.30	502.93	250, 199, 157	250	250 > 55, 250 > 200	250 > 200
Deltamethrin	47.38	505.24	253, 181, 172	253,172	253 > 172, 253 > 199, 172 > 93	172 > 93

can permit to monitor up to 200 transitions in the programmed time. In this study, the method development was focused on achieving in well separated chromatographic peaks with enough number of scans and adequate shape (Fig. 1).
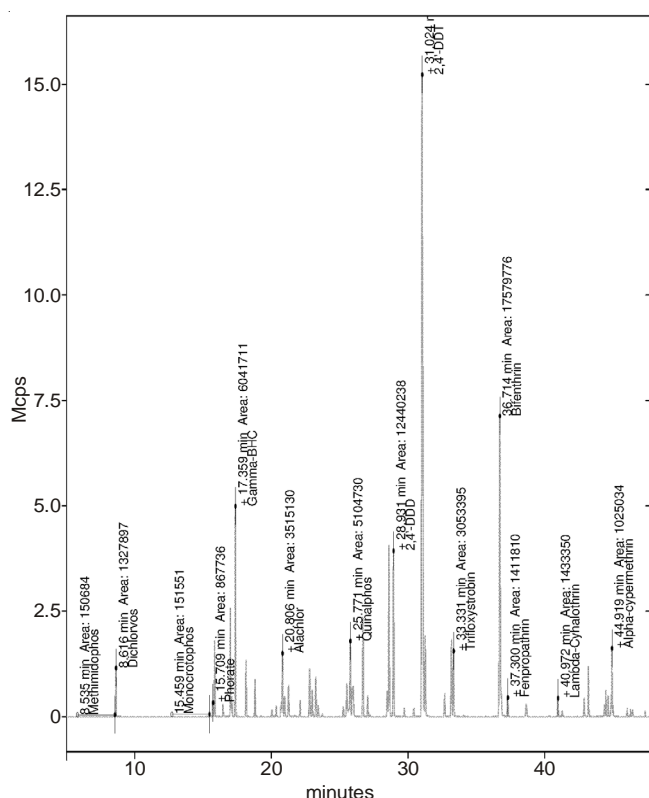

Fig. 1. Chromatogram of 64 mix pesticides at 0.5 µg/mL in multi reaction monitoring mode

Method validation: The proposed method was validated in order to ensure the reliability of the method for its application in daily laboratory analysis. Method validation experiment was done by using bitter gourd as representative sample matrix from the crop group with high water content[10].

The identification of the target compounds was based on relative retention time windows (RRTWs).The RRTWs were established as the relative retention time (RRT) average ± 3 times standard deviation (SD) of the retention time. Five blank samples were spiked at the second calibration level were injected (Table-1).

The quantification of the samples was done by matrix-matched standards calibration with concentration levels of 0.01, 0.025, 0.05, 0.075, 0.1 and 0.125 mg kg^{-1}. The concentrations of the calibration levels were selected for each pesticide according to the maximum residue limits (MRLs). The first calibration level was always equal or lower than the maximum residue limits established[11]. The linearity data obtained are shown in Table-2. Satisfactory linear values were obtained for all pesticides at proposed concentrations, the values are laid between 0.986- 0.999.

The limit of detection (LODs) was calculated by injecting lower pesticide concentration in blank matrix extract. The concentrations were 1 or 5 µg kg^{-1} for all pesticides in bitter gourd matrix. The limit of quantification (LOQs) were established as the first calibration point (10 µg kg^{-1}) after checking that the RSD percentage obtained from the injection of six calibration standards.

The accuracy of the method was calculated through the recovery of each pesticide. The recovery rate of each pesticide at three different fortification levels was evaluated in order to assess the extraction and cleanup efficiency of the proposed

TABLE-2
LINEARITY, RECOVERY AND LIMIT OF DETECTION (LODs) VALUES

Pesticides	Class	Linearity (R²)	0.01 Fortification level (mg kg⁻¹)		0.05 Fortification level (mg kg⁻¹)		0.1 Fortification level (mg kg⁻¹)		Limit of detection (mg kg⁻¹)
			*Recovery (%)	% RSD	*Recovery (%)	% RSD	*Recovery (%)	% RSD	
Methamidophos	OP	0.997	80.99	9.62	81.62	8.99	82.00	9.21	0.005
Dichlorvos	OP	0.994	84.62	7.88	83.69	6.42	84.12	5.82	0.005
Monocrotophos	OP	0.999	75.69	10.99	74.62	13.19	75.62	14.62	0.001
Phorate	OP	0.993	91.00	5.90	91.0	7.63	79.40	8.59	0.005
α-HCH	OC	0.999	76.60	16.96	83.70	13.87	79.60	17.36	0.001
Dimethoate	OP	0.996	112.50	15.63	85.60	8.61	130.0	15.33	0.001
β-HCH	OC	0.999	101.80	5.89	90.60	7.67	81.00	14.69	0.001
Atrazine	H	0.986	85.00	6.34	86.80	9.44	76.10	9.05	0.005
Lindane	OC	0.992	90.02	7.02	86.12	8.01	72.40	17.30	0.005
Chlorthalanil	OC	0.997	86.00	5.04	81.12	6.09	80.00	11.00	0.001
Diazinon	OP	0.998	82.06	9.02	84.14	8.03	81.00	8.02	0.005
δ-HCH	OC	0.997	81.00	5.42	81.11	7.01	83.07	5.01	0.005
Phophomidon	OP	0.995	87.12	6.12	86.00	4.12	85.00	5.62	0.001
Chlorpyrifos methyl	OP	0.991	83.09	8.10	82.02	7.99	83.12	6.22	0.005
Methyl parathion	OP	0.997	88.12	8.72	87.19	6.12	86.22	7.13	0.001
Alachlor	H	0.998	91.10	9.12	90.02	8.88	89.00	7.12	0.001
Heptachlor	OC	0.998	92.00	10.02	92.62	10.09	93.12	9.86	0.005
Metalaxyl	OC	0.994	89.00	6.66	89.72	7.00	89.00	8.01	0.005
Demeton-S-methyl sulfone	OP	0.997	81.02	7.12	82.12	8.00	83.02	7.19	0.005
Fenitrothion	OP	0.997	84.02	5.99	85.14	6.12	88.00	5.12	0.001
Malathion	OP	0.992	91.90	9.02	90.09	8.16	92.02	3.18	0.005
Aldrin	OC	0.997	87.00	7.00	86.89	5.66	86.04	4.99	0.001
Chlorpyrifos	OP	0.995	89.99	8.12	90.12	7.02	91.24	6.55	0.001
Fenthion	OP	0.992	85.22	6.66	84.99	5.44	86.02	7.02	0.005
Parathion	OP	0.996	80.02	4.12	80.99	5.12	81.42	4.00	0.001
Dicofol	OC	0.997	79.99	3.02	80.11	4.67	81.00	5.62	0.001
Dieldrin	OC	0.997	78.22	3.42	79.42	4.56	80.12	5.12	0.001
Fipronil	OP	0.992	83.42	5.55	84.02	6.16	85.04	7.01	0.005
Chlorfenvinphos	OP	0.997	86.14	3.92	87.00	4.64	88.62	5.01	0.001
Quinolphos	OP	0.993	87.88	4.04	87.86	5.12	88.80	6.02	0.005
Allethrin-a	SP	0.994	91.42	8.01	90.96	7.66	90.42	5.66	0.005
Allethrin-b	SP	0.998	90.24	7.62	90.88	8.11	90.99	7.62	0.005
2,4-DDE	OC	0.997	92.14	10.12	91.68	9.87	92.02	8.42	0.001
α-Endosulfan	OC	0.994	93.19	2.62	94.14	3.69	95.00	4.02	0.001
Butachlor	H	0.995	96.02	6.60	95.76	4.02	95.86	6.12	0.005
Hexaconazole	F	0.996	97.14	4.99	97.62	5.11	96.02	6.02	0.001
Fenamiphos	OP	0.996	82.12	6.19	83.66	7.02	84.02	6.92	0.005
Profenophos	OP	0.997	88.42	8.02	89.11	7.77	89.42	8.02	0.005
4,4-DDE	OC	0.999	81.02	9.04	82.62	8.89	84.10	6.44	0.005
2,4-DDD	OC	0.999	83.69	6.11	83.99	7.62	84.28	9.02	0.001
Endrin	OC	0.991	80.06	6.88	81.21	7.02	80.99	7.42	0.001
β-Endosulfan	OC	0.994	83.99	7.77	84.62	8.09	85.42	8.19	0.001
4,4-DDD	OC	0.996	86.92	8.12	84.09	7.77	89.02	7.09	0.001
2,4-DDT	OC	0.998	88.99	9.02	88.62	8.79	86.42	7.19	0.001
Ethion	OP	0.999	90.66	8.42	91.92	7.19	92.42	8.09	0.005
Triazophos	OP	0.991	81.62	10.42	81.92	10.99	83.09	11.24	0.005
Endosulfan sulphate	OC	0.994	89.09	7.86	89.99	8.42	87.64	9.42	0.001
4,4 DDT	OC	0.993	92.99	9.09	93.44	8.42	91.99	7.42	0.001
Trifloxystrobin	F	0.994	90.62	5.66	91.99	8.88	90.99	5.99	0.005
Tebuconazole	F	0.990	80.99	6.11	81.42	7.09	82.42	8.42	0.005
Bifenithrin	SP	0.994	94.62	5.02	92.19	6.17	93.48	5.99	0.001
Methoxychlor	OC	0.996	87.76	6.66	87.02	6.19	88.77	7.12	0.001
Fenpropathrin	SP	0.993	79.96	8.01	80.02	4.77	80.00	5.02	0.005
Phosalone	OP	0.998	86.66	7.62	89.09	6.88	82.92	5.88	0.005
λ-Cyhalothrin	SP	0.997	91.99	4.77	92.49	5.88	93.02	7.77	0.005
Azinphos ethyl	OP	0.994	81.09	8.42	80.09	6.99	80.99	5.97	0.005
Permethrin-I	SP	0.998	87.77	4.99	88.12	5.55	86.99	4.88	0.005
Permetrin-II	SP	0.997	85.99	5.98	84.99	6.11	85.87	7.01	0.005
Cyfluthrin	SP	0.992	90.12	9.99	87.62	10.09	88.00	11.02	0.005
Cypermethrin	SP	0.993	84.14	8.02	86.19	7.82	83.19	6.82	0.005

Pesticides	Class	Linearity (R^2)	0.01 Fortification level (mg kg⁻¹)		0.05 Fortification level (mg kg⁻¹)		0.1 Fortification level (mg kg⁻¹)		Limit of detection (mg kg⁻¹)
			*Recovery (%)	% RSD	*Recovery (%)	% RSD	*Recovery (%)	% RSD	
α-Cypermethrin	SP	0.996	80.42	5.14	81.69	5.55	88.49	7.89	0.005
Fenvalarate	SP	0.993	82.14	7.89	83.96	5.89	87.77	9.09	0.005
Fluvalinate-I	SP	0.991	88.16	5.62	86.69	5.80	81.86	2.09	0.005
Fluvalinate-II	SP	0.992	87.29	4.99	82.09	8.22	80.99	5.06	0.005
Deltamethrin	SP	0.998	90.06	5.04	91.88	4.62	91.99	6.88	0.005

*Average of three replications, OP= Organophosphates, OC= Organochlorines, SP= Synthetic pyrethroids, H= Herbicide, F= Fungicide

TABLE-3
DETECTED PESTICIDES IN REAL SAMPLES

Commodity	Number of samples analyzed	Detected pesticides	Number of positive samples	Concentration (mg kg⁻¹)
Ash gourd	5	ND	–	–
Bitter gourd	5	Chlorpyriphos	1	0.2
		Quinolphos	2	2.0
Little gourd	5	Trifloxystrobin	1	3.0
		Tebuconazole	1	0.9
		Profenophos	2	2.3
Cucumber	5	Deltamethrin	1	0.5
		Quinolphos	1	0.75
		Profenophos	1	4.0
Ridge gourd	5	Acephate	2	2.0
Bottle gourd	5	ND	–	–
Snake gourd	5	Ethion	1	3.1
Water melon	5	ND	–	–
Gherkin	5	Chlorpyriphos	1	1.0
		Acephate	1	0.1
Pumpkin	5	ND	–	–

ND= Not detected

method. For this, 15 g of uncontaminated bitter gourd samples were spiked with the corresponding volume of the multi-compound working standard solution at each fortification level (0.01, 0.05 and 0.1 mg kg⁻¹). Thus the recovery levels were properly validated within three concentration levels. Satis-factory results were found in three fortification levels, with recoveries between 76.60 to 119 % at 0.01, 81.0 to 120 at 0.05 and 72.40 to 130 at 0.5 mg kg⁻¹ respectively (Table-2). The evaluation of recovery at these concentrations ensured that accuracy of the method.

Application of the method to the real samples: The vali-dated method was applied to the analysis of 50 cucurbit samples which were collected from different markets of Andhra Pradesh and Telangana states, India. Bitter gourd matrix was kept as a reference matrix for high-water and high-acid content vegetable. Ten different verities of cucurbits such as ash gourd, bitter gourd, little gourd, cucumber, ridge gourd, bottle gourd, snake gourd, water melon, gherkin and pumpkin were analyzed. The detected pesticides are listed in Table-3. A total of 30 % of analyzed samples gave positive result. The pesticides which are more commonly detected were chlorpyriphos, quinolphos, profenophos, acephate in bottle gourd, little gourd, cucumber, ridge gourd and gherkin, where as one bitter gourd sample has contaminated with multi residues of chlorpyriphos and quinolphos. One snake gourd sample was detected with ethion residues. Trifloxystrobin and tebuconazole residues were detected in little gourd sample. Chlorpyriphos, quinolphos and profenophos pesticides were recommended on cucurbits

by the states of Telangana and Andhra Pradesh local agricul-tural university. The maximum residue limit values for these pesticides on cucurbits are not available.

Conclusion

The QuEChERS method was validated for the analysis of more than 64 pesticide residues in 10 verities of cucurbit. Bitter gourd matrices were kept as reference matrices with high-water and high acid content vegetable. The QuEChERS multi residue method will allows the reduction of cost and the time per the sample. The method has given the excellent recovery values even in the very low concentrations. The instrumental analysis was carried out by GC-QqQ-MS/MS mainly operating in multi reaction monitoring mode in an optimized and fast running time of 49.67 min. The developed method was vali-dated in order to ensure the reliability of the method for its routine use in the laboratory.

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