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Development of a Novel Method for Detection of 54 Pesticides by GCMS/MS-TQD

A. HARINATHA REDDY[1], N.B.L PRASAD[2], D. RAVEENDRANATH[3], B. RAMESH[4] and K. LAKSHMI DEVI[5]*

[1]Department of Biotechnology, JNTUA, Ananthapuramu.
[2]OTRI, JNTUA, Ananthapuramu.
[3]Department of Biotechnology, JNTUH, Hyderabad
[4]Department of Biochemistry, SV University, Tirupathi.
[5]Department of Biochemistry, SK. University, Ananthapuramu.
*Corresponding author: klakshmidevi2000@yahoo.co.in

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ABSTRACT

A multi residue method for detection of 54 pesticides by GCMS/MS-TQD (Brukers 436 GC) was developed to Standardise of Organochlorines (OCs), organophosphates (OPs), synthetic pyrethroids (SPs), herbicide and fungicide mixtures (supplied by Dr.Ehrenstorfer and sigma Aldrich). Seven different concentrations of the standards i.e., 0.001, 0.005, 0.010, 0.015, 0.020, 0.025 and 0.30 ppm were prepared and six replications were injected into GC-MS/MS TQD. Linearity curve was drawn for each pesticide and regression values were calculated. Regression values ranged from 0.991 to 0.999 and percentage of relative standard deviation is in between 0.29 and 6.14 for all OCs. For OPs regression values ranged from 0.991 to 0.999 and percentage of relative standard deviation is in between 0.31 and 6.02. For SPs regression value ranged from 0.991 to 0.999 and percentage of relative standard deviation is in between 0.58 and 8.24. For herbicides, fungicides and other pesticides regression value ranged from 0.994 to 0.999 and percentage of relative standard deviation is in between 0.77 and 3.79. A method for the extraction of 54 pesticides belonging to chemical classes recovered from vegetables like tomatoes was developed and validated. Mixtures of 54 pesticides amenable to gas chromatography were quantitatively recovered from spiked tomato were determined by using gas chromatography mass spectroscopy. The sample preparation approach is known as QuEChERS, which stands for "quick, easy, cheap, effective, rugged and safe". As expected, the results are excellent and showed an overall average of 98% recoveries with 10% RSD. The method involved extraction with acetonitrile, liquid–liquid partition with addition of NaCl followed by $MgSO_4$ and primary secondary amine (PSA) and the analyses were carried out with GC–MS equipment. It was a rapid, simple and cost effective procedure. The spiking levels for the recovery experiments were 0.001, 0.005, 0.01 and 0.1 mg kg^{-1}. Adequate pesticide quantification and identity confirmation were done. The per cent recoveries of OCs are in the range of 83.65%–102.92% in case of 0.001 mg kg^{-1}, 78.78–99.66% in case of 0.01 mg kg^{-1} and of 81.18–108.50% in case of 0.1 mg kg^{-1}. The per cent recoveries of OPs are in the range of 81.45–100.60% in case of 0.005 mg kg^{-1}, 81.27–103.72% in case of 0.01 mg kg^{-1} and 79.07–102.77% in case of 0.1 mg kg^{-1}. The per cent recoveries of SPs are in the range of 91.00–98.69% in case of 0.005 mg kg^{-1}, 76.45–110.29% in case of 0.01 mg kg^{-1} and 90.56–107.40% in case of 0.1 mg kg^{-1}. The per cent recoveries of herbicides and fungicides are in the range of 93.57–98.72% in case of 0.005 mg kg^{-1}, 87.34–97.53% in case of 0.01 mg kg^{-1} and 79.10–93.07% in case of 0.1 mg kg^{-1} respectively.

Key words: GC-MS/MS-TQD, Multiresidue Method, Organo phosphates, Organo chlorines, Synthetic pyrethroids, Fungicides, Herbicides,,pesticides mixtures.

INTRODUCTION

Pesticides can broadly be classified as insecticides, fungicides and herbicides. Insecticides are mainly OCs, OPs, SPs, herbicides, fungicides, and carbamates. Organochlorine compounds are synthetic organic insecticides that contain carbon, hydrogen, chlorine and sometimes oxygen. The essential structural feature of OCs is the presence of carbon-chlorine bond or bonds (Stimman *et al*., 1985). They are therefore also called chlorinated hydrocarbons.

The determination of pesticide residues in vegetables and tropical fruits is of great interest for many countries, especially India and South America, that base an important part of their economy on the exportation of fruits and vegetables, mainly tropical fruits. Restrictive legislation around the world is applied to tropical fruits and vegetables, which have to accomplish the maximum residue levels (MRLs).

Exporting of commodities like chilies and curry leaf and some of the vegetables was banned because pesticide residues were detected in the commodities. Hence it is necessary to develop methods for the analysis of the pesticide residues in such commodities by gas chromatography.

The aim of this work is to develop modified procedures for the analysis of Multi class pesticides and their metabolites by gas chromatography Mass Spectroscopy (Harinatha Reddy et al (2013), Steven J Lehotay et al (1995) and to choose procedures for the detection of these substances in agricultural samples like vegetables and fruits and environmental samples like water and soil.

QuEChERS is a novel sample preparation technique for pesticide multiresidue analysis that was developed between 2000 and 2002 and first reported in 2003 (Steven Lehothy et al., 2007). This method is accurate, high recoveries will be achieved for many pesticides in many matrices.

MATERIALS AND METHODS

Chemicals like *n*-hexane, acetone, and acetonitrile (HPLC grade) were purchased from Merck, USA, and were glassware distilled before use. Acetone was refluxed over potassium permanganate for 4 h and then distilled. Sodium chloride (NaCl), anhydrous sodium sulfate (Na_2SO_4), and anhydrous magnesium sulfate ($MgSO_4$) were procured from Merck Pvt. Ltd. India. Before use anhydrous sodium sulfate (Na_2SO_4) and anhydrous magnesium sulfate ($MgSO_4$) were purified with acetone and baked for at 600°C 4 h in a muffle furnace to remove possible phthalate impurities. Primary secondary amine (PSA) bondasil 40 ìm part 12213024 were purchased from Agilent. Pesticide standards were procured from Suppelco Sigma– Aldrich USA, Fluka Sigma–Aldrich, New Delhi, India.

Standards and sample collection

n-hexane (Excellar) and toluene (HPLC Grade) were obtained from Merck and used for the preparation of standards. The pesticide reference materials at high purity (e"98 %) were supplied Dr. Ehrenstorfer GmbH (Augsburg, Germany) and sigma aldrich. For optimization and validation of the method pesticides (OCs, OPs, SPs, herbicides, fungicides and other pesticides) mixture were prepared at the concentration 1ìg/ml dissolved in (1:1) hexane: toluene solution. The solution was stored in the dark at 4°C for the preparation of further dilutions.

Six different concentrations of each category (OCs, OPs, SPs, Herbicide and Fungicide mixture) of pesticides were prepared separately for building a calibration curve. Each concentration level was injected six times, and the calculated mean value was used as the calibration point. LOD (Limit of detection), LOQ (Limit of quantification) and % RSD values were also calculated for each pesticide.

Six calibration standard solutions (0.001, 0.005, 0.010 , 0.015, 0.020, 0.025 ppm) of OCs viz., alpha HCH, aldrin, dieldrin, beta HCH, gamma HCH, delta HCH, heptachlor, dicofol, 2,4 DDE, alpha endosulfan, 4,4 DDE , 2,4 DDD, beta endosulfan, 4,4 DDD, 2,4 DDT, 4,4 DDT, endosulfan sulphate and hexaconazole. Six calibration standard solutions (0.005, 0.010 , 0.015, 0.020, 0.025, 0.030 ppm) OPs like dichlorvos, monocrotophos, diazinon, phorate, methamidophos, dimethoate, methyl parathion, chloropyrifos methyl, fenitrothion, malathion, azinphos ethyl, triazophos, chloropyrifos, quinalphos, profenophos, phosphomidon, chlorfenvinphos, parathion, fenamiphos, ethion, phosalone, SPs like bifenthrin, fluvalinate, fenvala rate, fenpropathrin, deltamethrin, lambda cyhalothrin, cypermethrin, alpha cypermethrin, permethrin, cyfluthrin, herbicides, atrazine, alachlor, butachlor fungicides like, trifloxystrobin, fipronil of other insecticides were prepared by adding different volumes of the composite standard solution and injected on GC-MS/MS-TQD. Tomatoes were collected from the field of student farm, College of Agriculture, Professor Jayashankhar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India.

Extraction and cleanup

The collected fresh tomato sample (100 g) was chopped, and ground in warring blender. 15 g sample in triplicate was taken for multi-pesticide residue analysis by QuEChERS method. The sample was macerated and mixed with 30 ml acetonitrile and 3 g of NaCl and centrifuged at 2500 rpm. Then 9 g of sodium sulphate was added to remove water content, and vortexed for 10 min at 50 rpm on rotospin test tube mixer. The extract was centrifuged at 10,000 rpm for 10 min. Nine milliliter aliquot of the supernatant extract was cleaned with the mixture of 0.4 g PSA, 1.2 g anhydrous $MgSO_4$ and 10 mg of activated charcoal. The extract was again shaken at 50 rpm on a rotospin for 10 min and centrifuged at 10,000 rpm for 10 min. Two ml of the supernatant was collected and evaporated with a turbovap and finally made up to 1ml with hexane. One microliter of the clean extract was used for the multi pesticide (OCs, SPs, OPs and herbicides and fungicides) residues analysis.

The final samples were analyzed on Brukers 436 GCMS equipped with fused silica capillary column factor Four (30 mt × 0.25 mm ID) coated with 1% phenyl-methyl polysiloxane (0.25 ìm film thickness) using Brukers 5 ms column. General operating conditions were as follows: Column temperature program was initially hold at 90˚C for 3 min, increased to 150˚C @ 20˚C hold for 6 min, increased to 220˚C @ 20˚C hold for 5 min, increased to 280˚C @ 50˚C/min hold for 5 min, Total 63 min. Injection volume 1 ìl; nitrogen flow rate is 1 ml/min with split ratio 1:10, using carrier gas helium 99.9%, Injector port temperature is 260°C, Detector parameters are Source- Triple Quadruple, Mass Range -50-400, Transfer line temperature 250°C, Source temp -220°C, Manifold temperature 40°C.

RESULTS AND DISCUSSION

The working standards of 54 pesticides prepared from the individual standards. A method was developed for the 54 pesticides including retention time, quantifier and qualifier ions are given in Table (1). Six point linearity curve was drawn by injecting OC mix, OP mix, SP mix, herbicide and fungicide mix. Regression values were also calculated from linearity for each pesticide. All the concentrations mentioned above were injected six times in order to calculate the regression value and % RSD values given in table (2).

In OCs, alpha HCH , aldrin, dieldrin, beta HCH, gamma HCH, delta HCH, heptachlor, dicofol, 2,4 DDE, alpha endosulfan 4,4 DDE ,2,4 DDD, beta endosulfan, 4,4 DDD, 2,4 DDT, 4,4 DDT, endosulfan sulphate and hexaconazole showed regression values ranging from 0.991to 0.999 and percentage of relative standard deviation is in between 0.29 and 6.14 Table 2). LOD of OC pesticides were 0.001 mg kg^{-1} similarly, the per cent recoveries of OCs are in the range of 83.65% to 102.92% in 0.001 mg kg^{-1}, in the range of 78.78 to 99.66% in 0.01 mg kg^{-1} and in the range of 81.18 to 108.50% in 0.1 mg kg^{-1} respectively (Table 3).

In OPs, Dichlorvos, monocrotophos, diazinon, phorate, methamidophos, dimethoate, methyl parathion, chloropyrifos methyl, fenitrothion, malathion, azinphos ethyl, triazophos, chloropyrifos, quinalphos, profenophos, phosphomidon, chlorfenvinphos, parathion, fenamiphos, ethion, phosalone shows the regression value ranged from 0.991 to 0.998 and percentage of RSD is between 0.31 and 6.02 (Table 2). LOD of OP pesticides were 0.005 mg kg^{-1}. Similarly, the per cent recovery of OPs is in the range of 81.45 to 100.60% in 0.005 mg kg^{-1} in the range of 81.27 to 103.72% in 0.01 mg kg^{-1} and in the range of 79.07 to 102.77% in 0.1 mg kg^{-1} respectively (Table 3).

In SPs, bifenthrin, fluvalinate, fenvalarate,

Table 1: Method development for the 54 pesticides in GC- MS/MS-TQD

Compound	Retention Time	Molecular Weight	Monitoring Ions	Precursor Ion	Qualifier Ion	Quantifier Ion
Dichlorvos	7.70	220.98	237, 235	185	185>63, 185>93, 185>109	185>93
Monocrotophos	17.48	223	192, 127, 164	127	127>109, 127>95, 127>79	127>109
Phorate	17.92	276	260, 231, 121	260	260>175, 260>231, 121>93	121>93
Alpha HCH	18.14	290.82	219, 181, 183	219, 181	219>183, 219>147, 181>145	181>145
Dimethoate	19.20	229.28	125, 229, 93, 87	125, 229	125>79, 125>93, 125>125, 125>87	125>125
Beta HCH	20.12	290.82	219, 181, 183	181, 219	181>145, 219>183	181>145
Atrazine	20.11	215.68	215, 200	215	215>200, 215>172, 215>138	215>200
Lindane	20.30	290.8	181, 219, 183	181, 219	181>145, 219>183	181>145
Diazinon	21.40	304.3	304, 779, 179	304, 179	304>137, 304>164, 304>179, 179>137	179>137, 304>137
Methamidophos	21.87	141.34	141, 94	141	141>64, 141>79, 141>95	141>95
Delta HCH	22.66	290.82	219,183, 181	181, 219	181>145, 219>183	181>145
Chlorpyrifos methyl	24.93	322.53	286, 125	286	286>208, 286>241	286>241
Methyl parathion	25.42	263.21	263, 223, 125	263	263>109, 263>127, 263>246	263>109
Alachlor	25.57	269.76	188, 369, 238, 240	188, 269	188>160, 188>130, 269>160, 269>188	188>160, 269>160
Heptachlor	25.64	373.32	337, 274, 272	272	272>237, 272>141, 272>117	272>237
Fenitrothion	27.43	277	277, 260	260,277	260>109, 260>125, 260>151, 277>109, 277>260	260>109, 277>109
Malathion	28.18	330.36	173, 127, 125	173	173>99, 173>117, 173>127	173>99
Aldrin	28.42	364.91	263,286, 314, 293	263	263>193, 263>228	263>193
Chlorpyrifos	28.81	350.62	314, 286, 197	314,286	314>166, 314>258, 314>286, 286>93, 286>271	314>258
Phophomidon	29.09	299	264, 127	264	264>72, 264>127, 264>193	264>127
Parathion	29.29	291.3	291, 261, 235	291	291>109, 291>137	291>109
Dicofol	30.16	370.48	250, 251, 759	251	251>139, 251>111	251>139
Dieldrin	36.90	380.9	277,263	277,263	277>241,277>206,277>170,263>193, 263>228	263>193
Fipronil	32.36	437.15	367, 369, 351, 213	367	367>178, 367>213, 367>255	367>213
Chlorfenvinphos	32.51	359.57	323, 267	267, 323	267>159, 323>267	323>267
Quinolphos	32.84	298	298, 146, 157, 118	298, 146, 157	298>129, 298>156, 298>190, 146>118, 157>129	146>118

2,4 DDE	34.14	318.03	237, 235	246,318,163, 226	246>176,318>318,318>246, 163>127, 226>206	246>176
Alpha endosulfan	34.88	406.93	241, 265, 277, 243	241, 265	241>206, 241>170, 265>229, 265>195, 265>193	241>206
Butachlor	35.08	311.9	237, 323, 240, 266	237, 323	237>160, 237>188, 176>134, 176>146, 188>130	176>146
Hexaconazole	36.28	314.21	214, 175	214	214>124, 214>152, 214>172	214>172
Fenamiphos	35.75	303.3	303,288, 154	303	303>139, 303>154, 303>180	303>154
Profenophos	38.69	372	339, 139, 559, 759	339, 139	339>188, 339>251, 339>269, 139>97	139>97
4,4 DDE	36.98	318.03	318, 246	318, 246	318>176, 318>246, 246>176, 318>318	318>318
2,4 DDD	40.94	320.05	237, 235	235	235>165, 235>200, 235>139	235>165
Beta endosulfan	39.90	406.93	241, 195	195, 241	195>159, 241>206	195>159
4,4 DDD	40.94	320.05	237, 235	235	235>165, 235>199, 235>200	235>165
2,4DDT	40.57	354.49	237, 235	235, 141	235>200, 235>235, 141>95	141>95
Ethion	40.84	384.48	231, 384, 257, 153	231	231>129, 231>175, 231>203	231>129
Phosalone	40.84	367	367, 182	367, 182	367>111, 367>138, 367>182, 182>138, 182>111	367>111, 182>111
Triazophos	42.65	313	257, 161	257	257>119, 257>134, 257>162	257>162
Endosulfan sulphate	43.44	422.92	274, 272, 387	272, 387	272>141, 272>165, 272>237, 387>253	272>237
4,4 DDT	44.08	354.49	237, 235	235	235>165, 235>199, 235>200, 235>235, 235>199	235>165
Trifloxystrobin	44.28	408.37	222, 116, 190	222, 116, 190	222>190, 222>162, 222>130, 116>89, 190>130	116>89
Bifenithrin	49.73	422.87	181, 165, 166	181, 165	181>115, 181>165, 181>166, 165>115	181>166
Fenpropathrin	50.36	349	265, 165, 181,125	265,165,181	265>210, 265>181, 165>153, 181>152	181>152
Lambda cyhalothrin	50.86	449.9	181, 797	181, 797	181>127, 181>152	181>152
Azinphos ethyl	53.06	345.4	160, 134, 155, 127	160,134,155, 127	160>102, 160>105, 160>132	160>132
Permethrin-I	55.79	390	183, 163	163, 183	163>127, 183>153	183>153
Permetrin-II	55.77	390	183, 163	163, 183	163>127, 183>153	163>127
Cyfluthrin	57.70	434.3	226, 206, 163	206, 163, 226	206>151,206>177,206>179, 163>127, 226>206	206>177
Cypermethrin	57.92	416.32	163, 181, 165, 127	163, 181	163>127, 181>152	163>127
Alpha cypermethrin	58.17	406.93	241, 265, 277, 243	241, 265	241>206,241>170,265>229, 265>195, 265>193	241>206
Fenvala rate	60.28	419	225, 167	225	225>91, 225>119, 225>147	225>119
Fluvalinate-I	60.65	502.93	250, 199, 157	250	250>55, 250>200	250>200
Fluvalinate-II	60.67	502.93	250, 199, 157	250	250>55, 250>200	250>200
Deltamethrin	62.9	505.24	253, 181, 172	253,172	253>172, 253>199, 172>93	172>93

Table 2: Pesticide correlation coefficient and relative standard deviation values

S. No	Name of the Standard	Correlation coefficient (R^2) values	RSD
1.	Dichlorvos	0.996	1.81-3.21
2.	Methamidophos(1ppm)	0.992	2.54- 3.35
3	Monocrotophos(1ppm)	0.991	2.43- 5.11
4	Phorate	0.993	2.99- 4.01
5.	Alpha HCH	0.992	1.01- 2.99
6.	Dimethoate	0.994	0.31-3.56
7.	Beta HCH	0.991	1.62- 1.99
8.	Atrazine	0.998	1.01-3.34
9.	Lindane	0.999	2.01- 3.01
10.	Diazinon	0.998	0.49-2.09
11.	Delta HCH	0.995	1.89- 2.21
12.	Phophomidon	0.997	0.93- 2.35
13.	Chlorpyrifos methyl	0.998	1.79- 4.07
14.	Methyl parathion	0.996	3.39- 4.24
15.	Alachlor	0.997	1.42- 3.57
16.	Heptachlor	0.998	0.85- 2.37
17.	Fenitrothion	0.995	2.09- 5.81
18.	Malathion	0.995	2.08- 3.99
19.	Aldrin	0.996	2.01- 3.54
20.	Chlorpyrifos	0.997	1.04- 3.70
21.	Parathion	0.999	2.28- 4.11
22.	Dicofol	0.996	1.18- 2.42
23.	Dieldrin	0.999	0.66-3.33
24.	Fipronil	0.995	1.12- 2.75
25.	Chlorfenvinphos	0.997	1.32- 2.01
26.	Quinalphos	0.998	0.32- 1.35
27.	2,4 DDE	0.998	0.76- 3.09
28.	2,4 DDT	0.997	1.44- 3.09
29.	4,4 DDE	0.998	2.31-4.98
30.	Alpha endosulfan	0.995	1.04- 3.24
31.	Butachlor	0.999	0.88- 2.34
32.	Hexaconazole	0.999	1.23- 3.79
33.	Fenamiphos	0.997	1.22- 3.64
34.	Profenophos	0.998	2.58- 6.02
35.	2,4 DDD	0.998	1.54- 2.78
36.	Beta endosulfan	0.999	1.54- 4.92
37.	4,4 DDD	0.999	1.04- 3.22
38.	Ethion	0.996	2.11- 2.56
39.	Triazophos	0.997	1.34- 2.21
40.	Endosulfan sulphate	0.998	0.29- 6.14
41.	4,4 DDT	0.997	1.03- 3.10
42.	Trifloxtstrobin	0.998	1.23- 3.19
43.	Bifenithrin	0.998	1.04- 3.11
44.	Fenpropathrin	0.997	1.59- 3.84
45.	Phosalone	0.995	1.06- 2.19
46	Lambda cyhalothrin	0.995	1.04- 3.56

47	Azinphos ethyl	0.994	1.09- 2.23
48	Permethrin –I	0.991	1.33- 3.28
49	Permetrin-II	0.994	1.58- 2.31
50	Cyfluthrin	0.996	0.58- 3.23
51	Cypermethrin	0.997	1.35- 1.89
52	Alpha cypermethrin	0.998	2.41- 4.99
53	Fenvala rate	0.999	1.78-5.54
54	Fluvalinate-I	0.997	1.81-8.24
55	Fluvalinate-II	0.997	2.77- 5.91
56	Deltamethrin	0.996	2.12- 4.89

Table 3: Fortification and recovery in tomato in the level of 0.001-0.005 mg kg^{-1}, 0.01 mg/kg^{-1}, 0.1 mg/kg^{-1}

S. No	Name of the standard	Recovery values in Tomato			
		0.001-0.005mg/kg	0.01mg/kg	0.1mg/kg	LOD
1.	Dichlorvos	97.27	99.75	97.22	0.005
2.	Methamidophos(1ppm)	85.40	86.49	88.60	0.005
3.	Monocrotophos(1ppm)	92.00	94.56	97.03	0.005
4.	Phorate	88.58	93.64	88.58	0.005
5.	Alpha HCH	98.84	97.12	90.02	0.001
6.	Dimethoate	96.16	81.99	93.90	0.005
7.	Beta HCH	101.81	95.94	82.76	0.001
8.	Atrazine	95.65	93.36	79.10	0.005
9.	Lindane	93.99	99.53	87.01	0.001
10.	Diazinon	86.84	103.72	80.44	0.005
11.	Delta HCH	93.95	88.00	90.62	0.001
12.	Phophomidon	96.06	93.89	95.60	0.005
13.	Chlorpyrifos methyl	98.52	99.01	94.31	0.005
14.	Methyl parathion	89.19	98.08	90.41	0.005
15.	Alachlor	83.65	90.98	96.15	0.005
16.	Heptachlor	98.66	86.00	83.51	0.001
17.	Fenitrothion	91.58	84.10	88.54	0.005
18.	Malathion	88.71	81.27	79.07	0.005
19.	Aldrin	102.92	99.66	84.64	0.001
20.	Chlorpyrifos	87.25	93.87	86.98	0.005
21.	Parathion	95.04	87.93	96.29	0.005
22.	Dicofol	92.14	91.90	98.56	0.001
23.	Dieldrin	96.77	94.88	93.76	0.001
24.	Fipronil	97.37	90.00	81.85	0.005
25.	Chlorfenvinphos	88.78	84.96	80.08	0.005
26.	Quinalphos	90.49	97.44	85.65	0.005
27.	2,4 DDE	99.74	92.62	81.18	0.001
28.	Alpha endosulfan	94.11	86.74	89.55	0.001
29.	Butachlor	98.72	97.53	93.07	0.005
30.	Hexaconazole	98.00	91.30	86.29	0.001
31.	Fenamiphos	88.96	89.67	84.89	0.005
32.	Profenophos	89.02	85.79	88.23	0.005
33.	4,4 DDE	93.84	92.74	93.70	0.001

34.	2,4 DDD	96.65	97.51	88.61	0.001
35.	Beta endosulfan	94.12	88.16	91.89	0.001
36.	4,4 DDD	89.25	92.28	87.27	0.001
37.	2,4 DDT	90.41	78.78	84.76	0.001
38.	Ethion	100.60	88.51	91.23	0.005
39.	Triazophos	91.34	95.34	84.56	0.005
40.	Endosulfan sulphate	95.52	85.33	104.55	0.001
41.	4,4 DDT	86.92	83.40	108.50	0.001
42.	Trifloxtstrobin	93.57	87.34	84.52	0.005
43.	Bifenithrin	94.50	110.00	97.29	0.005
44.	Fenpropathrin	94.89	76.45	101.32	0.005
45.	Phosalone	99.34	93.56	85.27	0.005
46.	Lambda cyhalothrin	83.23	85.87	84.29	0.005
47.	Azinphos ethyl	81.45	82.95	83.56	0.005
48.	Permethrin –I	98.69	88.30	107.40	0.005
49.	Permetrin-II	98.67	84.06	107.00	0.005
50.	Cyfluthrin	92.37	95.27	103.35	0.005
51.	Cypermethrin	97.67	105.23	89.56	0.005
52.	Alpha cypermethrin	91.24	92.32	90.94	0.005
53.	Fenvala rate	91.00	110.29	92.57	0.005
54.	Fluvalinate-I	93.45	91.22	90.56	0.005
55.	Fluvalinate-II	93.11	91.02	88.11	0.005
56.	Deltamethrin	98.59	105.16	94.62	0.005

fenpropathrin, deltamethrin,, lamda cyhalothrin, cypermethrin, alpha cypermethrin, permethrin, cyfluthrin showed the regression value ranged from 0.991 to 0.999 and % of RSD is between 0.58 and 8.24 (Table 2) Similarly, the per cent recovery of SPs is in the range of 91.00 to 98.69% in 0.005 mg kg^{-1} in the range of 76.45 to 110.29% in 0.01 mg kg^{-1} and in the range of 90.56 to 107.40% in 0.1 mg kg^{-1} respectively (Table 3).

Herbicides and fungicides alachlor, butachlor, atrazine fungicides trifloxystrobin, fipronil of other insecticide showed the regression value ranging from 0.994 to 0.999 and percentage of relative standard deviation is between 0.77 and 3.79 (Table 2). Similarly, the per cent recovery of herbicides and fungicides is in the range of 93.57 to 98.72% in 0.005 mg kg^{-1}, in the range of 87.34 to 97.53% in 0.01 mg kg^{-1} and in the range of 79.10 to 93.07% in 0.1 mg kg^{-1} respectively (Table 3).

The use of acetone in place of acetonitrile in QuEChERS method has many advantages, but it has low recovery compared to acetonitrile and also it is difficult to analyze in LC. The use of acetonitrile in QuEChERS method has shown good recovery including its ability to separate from water upon the

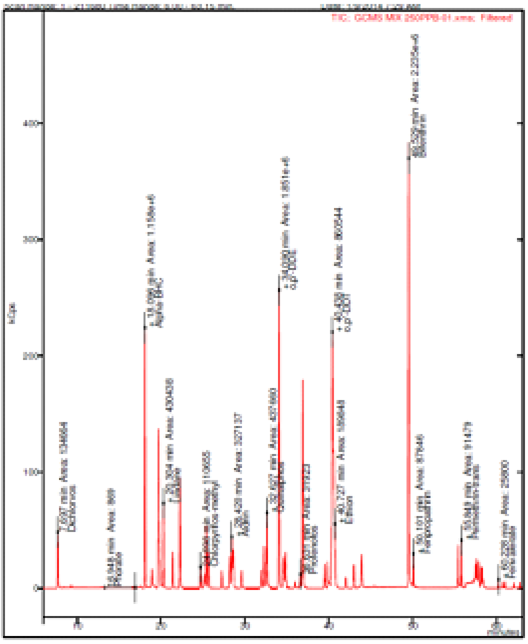

Fig. 1: OC, OP, SP, Herbicide and fungicides Standard mixture 250 ppb

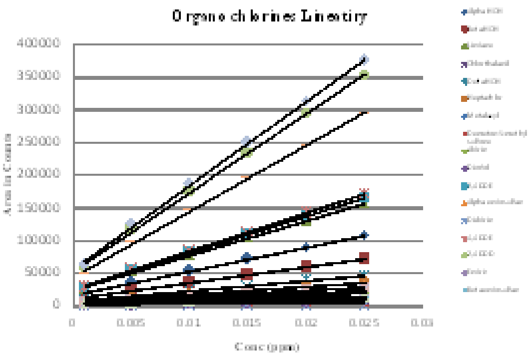

Fig. 2: Linearity curve for Organo chlorines

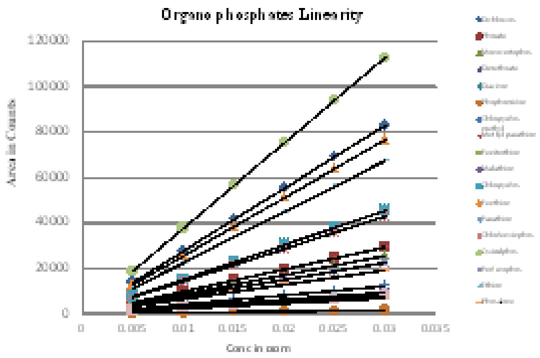

Fig. 3: Linearity curve for Organo phosphates

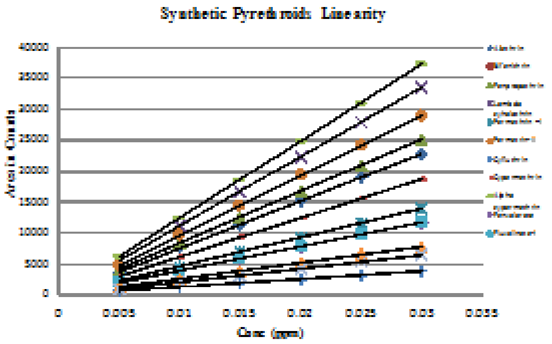

Fig. 4: Linearity curve for Synthetic Pyrethroids

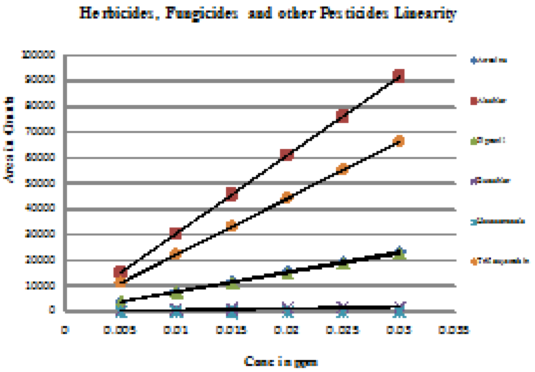

Fig. 5: Linearity curve for Herbicides, Fungicides and other pesticides

addition of salt without the addition nonpolar solvent and amenability with GC and LC applications.

Mixtures of 17 OCs at 6 different levels six times injected in to GC-MS for drawing the linearity curve showed regression values ranging from 0.991 to 0.999 and percentage of relative standard deviation is between 0.29 and 6.14 for the all OCs fig 2.

Mixture of 21 OPs at 6 different levels injected 6 times in to GC-MS for drawing linearity curve showed regression values ranging from 0.991 to 0.999 and percentage of relative standard deviation is between 0.31 and 6.02 for all the OPs fig 3.

Mixture of 10 SPs at 6 different levels injected 6 times in to GC-MS for drawing linearity curve showed regression values ranging from 0.991 to 0.999 and percentage of relative standard deviation is between 0.58 and 8.24 for all the synthetic pyrethroids fig. 4.

Mixture of herbicides and fungicides at 6 different levels injected 6 times in to GC-MS for drawing linearity curve showed regression values ranging from 0.994 to 0.999 and percentage of relative standard deviation is between 0.77 and 3.79 for all the herbicides and fungicides and other pesticides fig. 6.

CONCLUSION

A Multi residue method for determination of 54 pesticides of different categories viz., OCs, OPs, SPs, herbicide and fungicide mix was developed. All the pesticides were separated and could be analyzed by this single method. Linearity curve with all the regression values e" 0.940 and % RSD values in permissible range from 0.29 to 8.24 were obtained.

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