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Development of Novel Method for Detection of 38 Pesticides

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Developed multi residue method for 38 pesticides on GC-ECD Shimadzu 2010. Organochlorines, organo phosphates, synthetic pyrethroids mixtures of standards supplied by Ehrensortfer were prepared in (1:1) hexane:toluene. Eight different concentrations of standards *i.e.*, 0.005, 0.01, 0.025, 0.050, 0.075, 0.1, 0.25 and 0.50 ppm were prepared and injected six replications into GC-ECD. Linearity curve was drawn for each pesticide and regression values were calculated. The regression values for organo-chlorines were found from 0.940 to 0.990 and % RSD values were in permissible range from 0.57 to 10.4. For organophosphates regression values were found from 0.992 to 0.998 range % RSD values were ranged from 0.66 to 14.02. For synthetic pyrethroids, regression values were ranged from 0.956 to 0.999 and % RSD values ranged from 1.1 to 8.9. For all the pesticides linearity curve with all the regression values ≥ 0.940 and % RSD values in permissible range from 5.7 to 14.02.

Keywords: GC-ECD, Multi residue method, Pesticides, Organochlorines, Organophosphates, Synthetic pyrethroids.

INTRODUCTION

Pesticides can broadly be classified as insecticides, fungicides and herbicides. Insecticides are mainly organo chlorines, organo phosphates, synthetic pyrethroids and carbamates. Organochlorine compounds are synthetic organic insecticides that contain carbon, hydrogen, chlorine and sometimes oxygen. The essential structural feature about organo chlorines is the presence of carbon-chlorine bond or bonds[1]. They are therefore also called chlorinated hydrocarbons.

Organo chlorine pesticides had been the mostly used Insecticides but they have now been replaced with organo phosphate insecticides because of their environmental persistency. The environmental persistency of OCS has led to the ban of most of them not only as agrochemicals to control pest attack in agriculture but also for the formulation of other pesticide products such as mosquito coils[2]. The recent Stockholm convention on persistent organic pollutants (POPs) has banned the use of most organ chlorines.

OCPs are characterized by high persistence, low polarity, low aqueous solubility and high lipid solubility (lipophilicity) and as a result they have a potential to bio accumulate in the food chain posing a great threat to human health and the environment globally[3]. Organo chlorines have been implicated in abroad range of adverse human health effects including reproductive failures and birth defects, immune system malfunction, endocrine disruptions and cancers[4]. Studies have shown that dichlorodiphenyltrichloroethane (DDT), dieldrin and polychlorinated biphenyls (PCBs) have endocrine disrupting capacities[5]. Similarly, epidemiological studies have suggested an etiological relationship between exposure to organochlorines and Parkinson's diseases[6].

OPPs are a class of organic compounds containing C–P or C–O–P, C–S–P and –N–P bonds. These pesticides have been used for control of spiders, mites, aphids, beetles, caterpillars, *etc*., on a wide variety of crops worldwide, because of their advantages of high effectiveness and low toxicity. Because of this widespread use, they have become the most common and persistent contaminants of products of agriculture in developing countries[8]. OPPs inhibit acetyl cholinesterase, the enzyme that hydrolyzes the neurotransmitter acetylcholine and, thus, terminates the transmission signal on postsynaptic cholinergic receptors[9,10]. They can reach the food chain in various ways and may, therefore, pose a risk to human health[11]. Thus, the development of analytical methods suitable for their surveillance in food is quite important.

The determination of pesticide residues in vegetables and tropical fruits is of great interest for many countries, especially

those from India, 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).

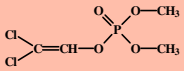
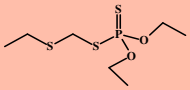
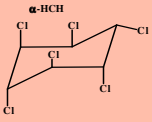
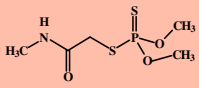
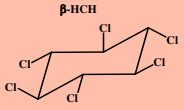
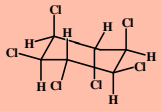
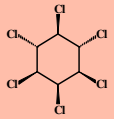
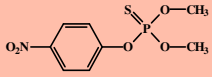
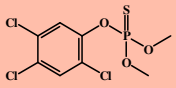
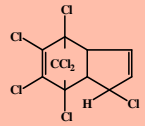
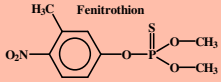
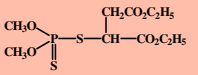
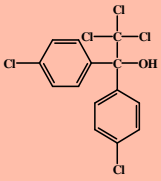
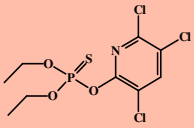
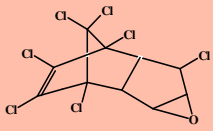
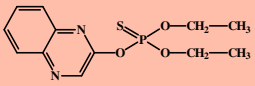
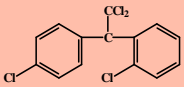
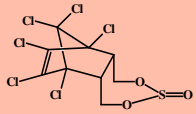
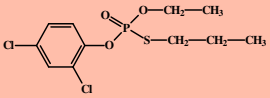
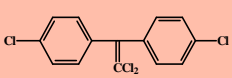
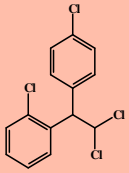
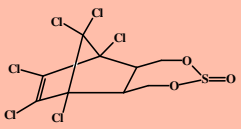
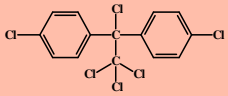
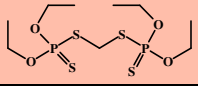
In exporting commodities like Chilies and Curry leaf and some of the vegetables banned the exporting, because the commodities were detected with the pesticide residues. Hence it is necessary to develop the methods to the analysis of the pesticide residues in gas chromatography.

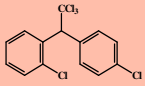
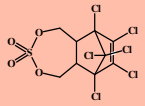
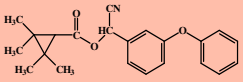
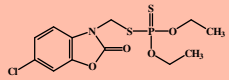
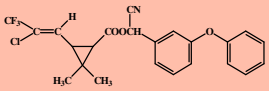
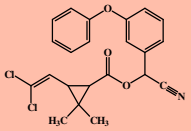
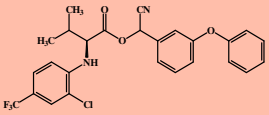
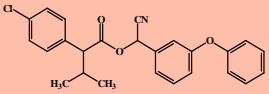
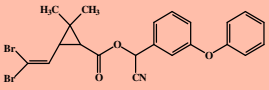
The aim of this work is to develop modified procedures for the analysis of pesticides[12-17] and its metobolites by gas chromatography and to choose procedures for the detection of these substances in agricultural samples like Vegetables and fruits and environmental samples like water, soil, respectively.

EXPERIMENTAL

n-Hexane (Excellar) and toluene (HPLC Grade) were obtained from Fischer Scientific and Merck respectively and used for the standards preparation. The pesticide reference materials at high purity (≥ 98 %, listed in Table-1) were supplied Dr. Ehrenstorfer GmbH (Augsburg, Germany). For method optimization and validation, pesticides mix (organo chlorines, organo phosphates and synthetic pyrethroids) were prepared at the concentration 1 µg/mL dissolved in (1:1) hexane:toluene solution. The solution was stored in the dark at 4 °C for the further dilutions.

TABLE-1
PESTICIDES (ORGANO CHLORINES, ORGANO PHOSPHATES AND SYNTHETIC PYRETHROIDS), CHEMICAL STRUCTURE, PURITY AND RETENTION TIME

S. No.	Name of the pesticide	Structure	Purity	Retention time
1	Dichlorvos		95.0	5.246
2	Phorate		98.0	18.2
3	α-HCH		98.0	18.3
4	Dimethoate		98.5	18.69
5	β-HCH		98.5	19.5
6	γ-HCH		98.6	20.4
7	δ-HCH		99.0	21.0
8	Methyl parathion		98.5	24.92
9	Chlorpyrifos methyl		98.5	25.20
10	Heptachlor		99.0	26.57
11	Fenitrothion		98.5	27.50
12	Malathion		99.0	28.78
13	Dicofol		98.5	29.89
14	Chlorpyrifos		98.5	29.91
15	Heptachlor exo epoxide		98.5	33.27
16	Quinalphos		99.0	33.86
17	2,4-DDE		99.0	36.08
18	α-Endosulfan		97.0	36.54
19	Profenophos		92.0	38.22
20	4,4DDE		98.5	39.07
21	2,4 DDD		98.5	39.50
22	β-Endosulfan		99.5	40.98
23	4,4-DDD		99.0	42.48
24	Ethion		98.8	42.80

25	2,4-DDT		98.0	43.16
26	Endosulfan sulfate		98.5	44.74
27	Fenpropathrin		99.0	51.69
28	Phosalone		98.5	53.23
29	λ-Cyhalothrin		98.5	56.23
30	Cypermethrin I		98.5	65.78
31	Cypermethrin II		98.5	66.59
32	Cypermethrin III		98.5	67.25
33	Cypermethrin IV		98.5	67.59
34	Fluvalinate I		98.5	73.48
35	Fluvalinate II		98.5	75.97
36	Fenvelarate I		98.0	77.06
37	Fenvelarate II		98.0	78.0
38	Deltamethrin		98.0	82.68

Six different concentrations of each category (organo chlorines, organo phosphates and synthetic pyrethroids) 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, LOQ and % RSD values were also calculated for each pesticide.

Six calibration standard solutions (0.005, 0.01, 0.025, 0.050, 0.075, 0.1, 0.25 and 0.5 ppm) of organo chlorines, α-HCH, β-HCH, γ-HCH, δ-HCH, heptachlor, dicofol, heptachlor exo epoxide, 2,4-DDE, α-endosulfan 4,4-DDE, 2,4-DDD, β-endosulfan, 4,4-DDD, 2,4-DDT, endosulfan sulphate. Organo phosphates dichlorvos, phorate, dimethoate, methyl parathion, chlorpyrifos methyl, fenitrothion, malathion, chloropyrifos, quinalphos, profenophos, ethion, phoslone, synthetic pyrethroids (SPs) fluvalinate, fenpropathrin, δ-methrin, λ-cyhalothrin, cypermethrin, permethrin, fluvalinate were prepared by adding different volumes of the composite standard solution and injected on GC ECD.

The final sample were analyzed on (Shimadzu) GC-2010 equipped with fused silica capillary column Factor Four (30 mt × 0.25 mm id) coated with 1 % phenyl-methyl polysiloxane (0.25 µm film thickness) using ^{63}Ni electron-capture detector (ECD). General operating conditions were as follows: Column temperature program: initially 130 °C for 5 min, increase at 3 °C/min to 180 °C hold for 5 min, then 240 °C increase 2 °C/min hold for 33.33 min; injection volume: 1 µL nitrogen flow rate 0.93 mL/min and makeup 25 mL/min with split ratio 1:10; using carrier gas (N_2) 99.5 %; injector port temperature 260 °C; detector temperature 300 °C.

RESULTS AND DISCUSSION

Eight point linearity curve (Figs. 1-3) was drawn by injecting organo chlorine mix, organo phosphate mix, synthetic pyrethroid mix. Regression values were also calculated from linearity for each pesticide. All the concentration mentioned above were injected six times in order to calculate % RSD values given in Tables 2-4.

It is seen that in Table-2, organo chlorine mix six concentrations injected six times and calculated the regression value and % RSD value. In organo chlorines, α-HCH regression value is 0.984 and % RSD range is 0.75-3.1, β-HCH regression value is 0.987 and % RSD range is 0.85-2.9, γ-HCH regression value is 0.982 and % RSD range is 1.01-4.0, heptachlor

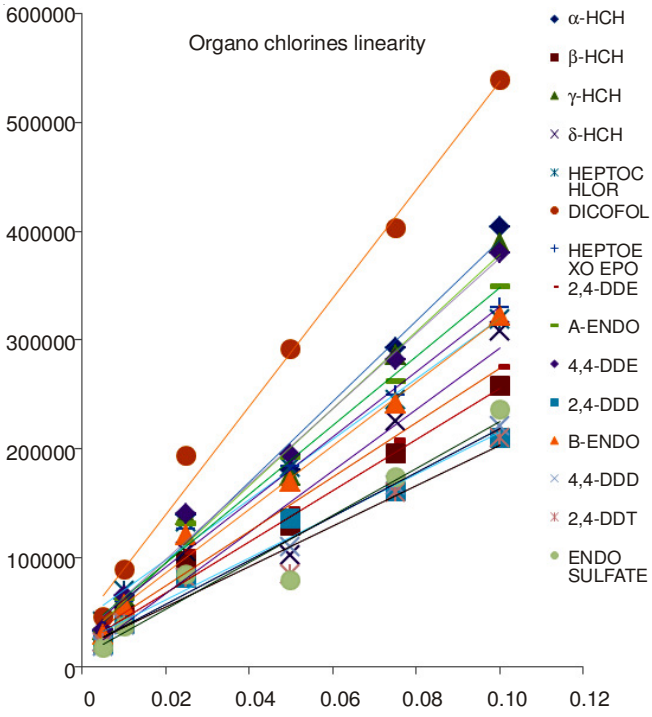


Fig. 1. Organo chlorines linearity

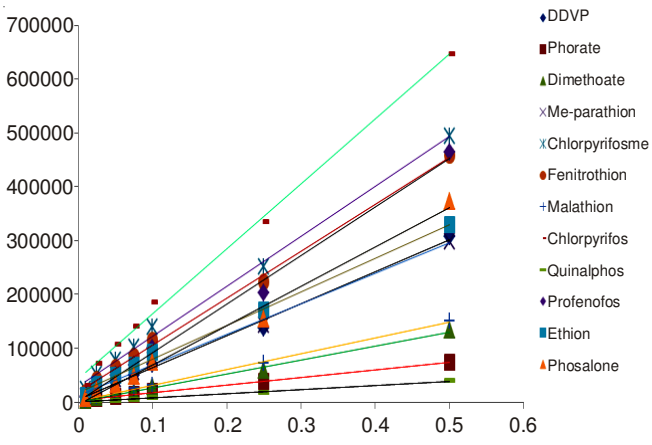


Fig. 2. Organo phosphates linearity

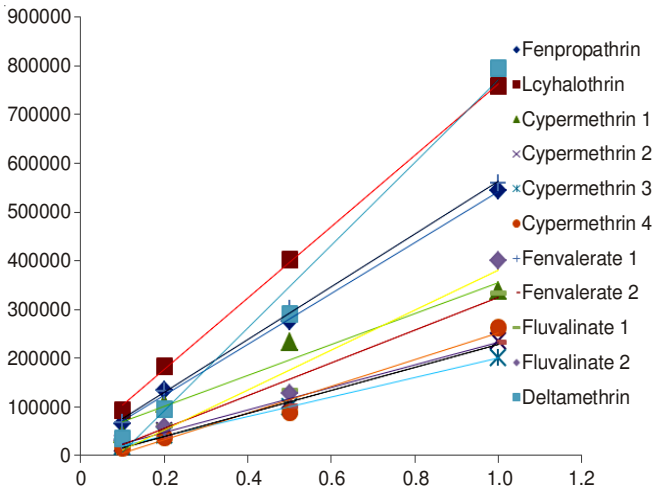


Fig. 3. Synthetic pyrethroids linearity

TABLE-2
ORGANO CHLORINE STANDARD AND REGRESSION VALUES AND PERCENT RELATIVE STANDARD DEVIATION VALUES

Standard name (OCS)	Regression values	% RSD
α-HCH	0.984	0.75-3.1
β-HCH	0.987	0.85-2.9
γ-HCH	0.982	1.01-4.0
δ-HCH	0.945	1.02-10.4
Heptachlor	0.987	1.05-2.7
Dicofol	0.992	1.04-3.5
Heptachlor exo epoxide	0.990	0.75-1.9
2,4-DDE	0.989	0.96-1.48
α-Endosulfan	0.989	0.96-1.48
4,4-DDE	0.990	0.77-2.2
2,4-DDD	0.989	1.01-1.86
β-Endosulfan	0.997	1.07-3.12
4,4-DDD	0.990	0.57-2.7
2,4-DDT	0.988	0.75-6.8
Endosulfan sulfate	0.958	0.57-5.05

TABLE-3
ORGANO PHOSPHATES STANDARD AND REGRESSION VALUES AND PERCENT RELATIVE STANDARD DEVIATION VALUES

Standard name (OPS)	Regression values	% RSD
Dichlorvos	0.995	3.83-5.30
Phorate	0.997	1.44-5.64
Dimethoate	0.994	2.12-6.53
Methyl parathion	0.995	1.39-5.16
Chlorpyrifos methyl	0.995	0.96-3.57
Fenitrothion	0.997	1.19-5.90
Malathion	0.998	1.94-5.90
Chlorpyrifos	0.995	1.94-9.70
Quinalphos	0.997	0.66-3.63
Profenophos	0.994	2.05-14.02
Ethion	0.995	2.05-8.23
Phosalone	0.992	1.08-6.65

regression value is 0.987 and % RSD range is 0.05- 2.7, dicofol regression value is 0.992 and % RSD range is 1.04-3.5, heptachlor exo epoxide regression value is 0.990 and % RSD range is 0.75-1.9, 2,4-DDE regression value is 0.989 and % RSD range is 0.96-1.48, α-endosulfan regression value is 0.990 and % RSD range is 0.77-2.2, 4,4-DDE regression value is 0.989 and % RSD range is 1.01-1.86, 2,4-DDD regression value is 0.997 and % RSD range is 1.07-3.12, β-endosulfan regression value is 0.990 and % RSD range is 0.57-2.7, 4,4-DDD regression value is 0.988 and % RSD range is 0.75-6.8, 2,4-DDT regression value is 0.958 and % RSD range is 0.57-5.05, endosulfan sulphate regression value is 0.940 and % RSD range is 1.48- 7.1 is given in Table-2 & Fig. 1.

It is seen that in Table-3 organo phosphate mix six concentrations injected by six times and calculated the regression value and % RSD value. In organo phosphates, dichlorvos regression value is 0.995 and % RSD range is 3.83-5.3, phorate regression value is 0.997 and % RSD range is 1.44-5.64, dimethoate regression value is 0.994 and % RSD range is 2.12-6.53, methyl parathion regression value is 0.995 and % RSD range is 1.39-5.16, chloropyrifos methyl regression value is 0.995 and % RSD range is 0.96-3.57, fenitrothion regression value is 0.997 and % RSD range is 1.19-5.9, malathion regression value is 0.998 and % RSD range is 1.94-5.90, chloropyrifos regression value is 0.990 and % RSD range is 1.94-9.70, quinolphos regression value is 0.997 and % RSD range is 0.66-3.63, profenophos regression value is 0.994 and % RSD range is 2.05-14.02, ethion regression value is 0.995 and % RSD range is 2.05-8.23, phosalone regression value is 0.992 and % RSD range is 1.08-6.65 is given in Table-3 & Fig. 2.

TABLE-4
SYNTHETIC PYRETHROIDS STANDARD AND REGRESSION VALUES AND PERCENT RELATIVE STANDARD DEVIATION VALUES

Standard name (SPS)	Regression values	% RSD
Fenpropathrin	0.998	1.19-1.92
L-Cyhalothrin	0.999	2.31-6.51
Cypermethrin 1	0.956	2.04-7.54
Cypermethrin 2	0.999	2.76-8.18
Cypermethrin 3	0.999	1.58-5.68
Cypermethrin 4	0.976	4.19-8.02
Fenvalarate 1	0.999	2.78-10.54
Fenvalarate 2	0.996	1.02-5.26
Fluvalinate 1	0.986	2.81-11.24
Fluvalinate 2	0.964	4.79-10.95
Deltamethrin	0.987	4.10-8.94

It is seen that in Table-4, synthetic pyrethroid mix six concentrations injected by six times and calculated the regression values and % RSD values were calculated. In synthetic pyrethroids, fenpropathrin regression value is 0.998 and % RSD range is 1.19-1.92, λ-cyhalothrin regression value is 0.999 and % RSD range is 2.31-6.51, cypermethrin 1 regression value is 0.956 and % RSD range is 2.04-7.54, cypermethrin 2 regression value is 0.999 and % RSD range is 2.76-8.18, cypermethrin 3 regression value is 0.999 and % RSD range is 1.58-5.68, cypermethrin 4 regression value is 0.976 and % RSD range is 4.19-8.02, fenvelarate 1 regression value is 0.999 and % RSD range is 2.78-10.54, fenvelarate 2 regression value is 0.996 and % RSD range is 1.02-5.26, fluvalinate 1 regression value is 0.986 and % RSD range is 2.81-11.24, fluvalinate 2 regression value is 0.964 and % RSD range is 4.79-10.95, δ-methrin regression value is 0.987 and % RSD range is 4.10- 8.94 is given in Table-4 & Fig. 3.

Mixtures of 15 organo chlorines[18] at 8 different levels six times were injected in to GC-ECD[19] for drawing the linearity curve, regression values were ranged from 0.940 to 0.999 %

RSD values were found in permissible range from 0.57 to 10.4 for the all organo chlorines.

Mixture of 12 organo phosphates at 8 different levels were injected 6 times in to GC ECD for drawing linearity curve. Regression values were found from 0.992-0.998 range % RSD values were in permissible times ranged from 0.66 to 14.02 for all the organo phosphates.

Mixture of 11 synthetic pyrethroids at 6 different levels were injected 4 times GC ECD for drawing linearity curve. Regression values were ranged from 0.956 to 0.999 and % RSD values ranged from 0.956 to 0.999 and % RSD values ranged from 1.1 to 8.9 for all the synthetic pyrethroid pesticides.

Conclusion

Multi residue method for determination of 38 pesticides of different categories *viz.*, organo chlorines, organo phosphates and synthetic pyrethroids has been developed. All the pesticides were separated and could be analyzed on single method. Linearity curve with all the regression values ≥ 0.940 and % RSD values in permissible range from 5.7 to 14.02 were calculated.

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