Pak. j. sci. ind. res., vol.37, no. 12, December 1994

A MULTI-RESIDUE METHOD FOR QUANTITATION OF ORGANOCHLORINE, ORGANOPHOSPHORUS AND SYNTHETIC PYRETHROID PESTICIDES IN COTTON SEEDS

ZAHIDA PARVEEN, IRSHAD A.K. AFRIDI* AND S.Z. MASUD

Pak. Agric. Research Council, Karachi University Campus, Old Block Nos 09 and 10, Karachi-75270, Pakistan

(Received September 25, 1993; revised August 16, 1994)

Analytical procedures have been developed for extraction, cleanup, identification and quantification of multiple residues of organochlorine, organophosphorus and synthetic pyrethroid pesticides in cotton seeds, collected from cotton growing areas of Punjab and Sindh provinces of Pakistan. These methods are efficient and reliable and allowed lipid removal to a large extent.

Key words: Multiple pesticide residues, Gas chromatography, Cottonseeds.

Introduction

Determination of trace levels of residues of multiple pesticides organochlorine (OC), organophosphorous (OP) and synthetic pyrethroid (SP) in cotton seeds containing high fat content is of particular interest. Hence, the first step is the extraction of the fatty (lipid) material from the homogenized sample. Liquid-liquid partioning between acetonitrile and *n*-hexane has been used in conjunction with column chromatography for proper cleanup of extracts. *n*-Hexane retained the pesticides while fats and other interfering components partitioned off in acetonitrile. The cleanup requirements differ from sample to sample or presence of specific physicochemical properties to separate the desired compounds from the sample extractives. Therefore the objective of the present work was to develop a cleanup procedure capable of handling fat and oil samples large enuogh to permit electron capture gas chromatographic determination of multiple pesticide residues approximately at ppb level in the cotton seeds within the shortest possible time. Numerous examples of extraction of different pesticide residues from fatty materials/cotton seeds are reported elsewhere [1-6]. Inefficient cleanup of sample causes rapid deterioration of gas chromatography (GC) system thereby precluding reliable results. For this reason, numerous applications of column chromatographic techniques using florisil, silica gel, alumina and gel permeation have been reported for the removal of fats and other substances interfering in solvent extracts from fatty materials [7-11]. The application of above mentioned methods does not assure quantitative determination of multi-residues of pesticides in cotton seeds. This paper reports the development of a methodology for complete recovery of OC, OP and SP residues from cotton seeds and minimizes or eliminates the difficulties encountered in other methods.

* Department of Chemistry, University of Karachi, Karachi.

Materials and Methods

All chemicals and solvents (AR grade and extra pure) for pesticide residue analyses were purchased from either Merck or BDH companies. The Pesticide Reference Standards (AR grade) procured from manufacturers (Table 1) were accurately weighed and dissolved in *n*-hexane or acetone. Individual standard solutions were diluted to exact concentration as needed for sample fortification, residue identification and quantification for recovery studies.

Extraction. Extraction mixture with double-distilled water + acetonitrile in the ratio of 1:4 was prepared and left overnight before use. Elution mixture of 15% diethyl ether in *n*-hexane was prepared and anhydrous sodium sulphate was added to remove moisture.

For fortification, three control samples of cotton seeds (5g) were ground for each experiment and transferred to a 250 ml conical flask. A calculated amount of each pesticide standard solution was separately added and followed by mixture of studied pesticides. The mixture were shaken for ten minutes, allowed to stand overnight at room temperature, and then processed for extraction and cleanup. A control sample of cotton seeds of the same quantity was also processed in a similar manner. For the extraction, 75 ml of the extraction mixture was added to the fortified sample and shaken with a mechanical shaker (Townson and Mercer Ltd.) for 3 hr. The extract was filtered through purified cotton wool, 10 g aluminium oxide and 5 g anhydrous sodium sulphate were then added, blended (Waring Commercial Blender) for 2 min at high speed and left at room temperature. After 15 min, the

supernatant liquid was filtered through Whatman filter paper No. 542.

n-Hexane partitioning. To the extract in acetonitrile, 75 ml of *n*-hexane was added and shaken for 30 sec in a separatory funnel. To this, 5 ml of saturated solution of sodium chloride and 50 ml of distilled water were added and the extracts were reshaken vigorously for 3 min. The layers were allowed to separate and the *n*-hexane layer was drained into a collecting flask. The process was repeated twice for complete extraction. Aqueous layers were collected and extracted with the same solvent, *n*-hexane retained the pesticides while fats and other interfering components remained in acetonitrile. *n*-Hexane layers were combined and concentrated in a rotary vacuum evaporator.

Clean-up. To achieve adequate cleanup of the extracts and good recovery of the pesticides, silica gel plus activated charcoal (as an adsorbent) were found to be the most suitable after a series of tests.

Preparation of chromatographic column. A clean and dry glass column (25 mm i.d. x 450 mm long) was plugged with cotton wool at the conical end and filled with 50 ml of *n*-hexane. A slurry of 5 g of activated silica gel was preparedwith 15% diethyl ether in *n*-hexane and poured into the column with continuous vibration to achieve compact packing. Excess solvents mixture was drained through the column and the mixture was allowed to settle. After all silica gel had settled, 10 g of activated silica gel (activated at 120°C for 3 hr.) and 1g of activated charcoal were mixed thoroughly and slowly stirred in eluting mixture. With constant stirring, this mixture was poured slowly and then in one rapid movement onto the silica gel layer in the column, under continuous vibration to achieve compact packing. During, this process the stopcock was left open. The eluting solvent mixture was drained until its surface was approximately 2 cm above the column packing. The top of the column packing was then covered with 5 g sodium sulphate.

Standardization of silica gel charcoal column. Three mixtures of the standards for the (OC, OP and SP) pesticides commonly used on cotton crop, were prepared in *n*-hexane. The known quantities were then transferred to the columns separately and allowed to settle for 5 min. The column was eluted with 15% diethyl ether in *n*-hexane. The flow rate was adjusted to 0.5 ml/min. Fractions of 100, 50, 50, 25 and 25 ml were collected and concentrated to a suitable volume for gas liquid chromatographic (GLC) analysis. A suitable aliquot 0.2-1 µl for electron capture detector (ECD) and 0.2-2 µl for thermionic specific detector (TSD) of each concentrated fraction was injected into the gas chromatograph to determine the recovery of each pesticide. All the studied pesticides were eluted in the 250 ml eluant. The extract of a control sample was

TABLE 1. RETENTION TIMES OF STUDIED PESTICIDES ON TWO DIFFERENT COLUMNS USING ELECTRON CAPTURE DETECTOR.

Pesticide	Retention time (Min)	
	Column-I	Column-II
Aldrin	1.681	2.139
γ-BHC	0.872	1.550
Bifenthrin	5.491	7.241
Chlorpyrifos	1.867	2.152
Cyfluthrin	19.488	23.596
Cyhalothrin	10.979	13.789
Cypermethrin	22.00	27.721
Deltamethrin	30.00	28.327
Diazinon	2.086	3.446
Dicofol	9.869	5.869
Dieldrin	3.183	3.734
Dimethoate	0.879	1.660
o,p'-DDE	2.472	3.023
o,p'-DDD	3.323	4.041
o,p'-DDT	3.771	4.861
p,p'-DDT	4.695	7.550
Endosulfan	4.413	3.998
Fenpropathrin	7.341	11.505
Fenvalerate	29.021	25.140
Flucythrinate	11.254	24.010
Fluvalinate	26.782	19.731
Heptachlor	0.874	1.928
Methamidophos	0.869	2.139
Monocrotophos	0.876	1.910
Parathion-methyl	2.014	1.864
Permethrin	13.247	18.230
Pirimiphos-methyl	0.895	1.321
Profenofos	3.346	4.693

TABLE-2. RETENTION TIMES OF STUDIED PESTICIDES ON TWO DIFFERENT COLUMNS USING THERMIONIC SPECIFIC DETECTOR.

Pesticide	Retention time (Minutes)	
	Column-I	Column-II
Chlorpyrifos	4.444	5.016
Cyhalothrin	14.786	14.069
Cypermethrin	30.279	N.D.
Deltamethrin	N.D.	N.D.
Dimethoate	4.091	4.652
Endosulfan	7.695	6.789
Fenvalerate	30.032	27.728
Fluvalinate	N.D.	N.D.
Methamidophos	1.321	1.621
Monocrotophos	3.224	4.027
Parathion-methyl	2.712	3.672
Pirimiphos-methyl	4.566	5.796

also processed to assess the efficiency of silica gel + activated charcoal to remove plant coextractives including oil components. It was found that 15g of activated silica gel and 1g of activated charcoal were enough for this cleanup.

Clean-up of fortified sample extract. The concentrated extract of the fortified sample (Table 3) in *n*-hexane was quantitatively transferred to the prepared column and allowed to adsorb into the column bed for 10 min. It was eluted with the elution mixture at the rate of a 0.5 ml/min. Eluant (250 ml) was collected for each sample. Each eluate was dried under vacuum at 40° and then added to it an appropriate quantity of *n*-hexane for gas chromatography.

TABLE 3. RECOVERY OF STUDIED PESTICIDES FROM 5G COTTON SEED SAMPLES ON TWO DIFFERENT GLC COLUMN MATERIALS.

Pesticide	Fortification level ppm (mg kg^{-1})	%Recovery*	
		Column-1	Column-2
Aldrin	0.001	93.9 ± 0.49	93.5 ± 0.53
γ-BHC	0.001	97.5 ± 0.48	97.4 ± 0.63
Bifenthrin	0.010	88.3 ± 1.39	88.5 ± 0.49
Chlorpyrifos	0.001	98.3 ± 0.83	98.0 ± 0.38
Cyfluthrin	1.0	99.4 ± 1.01	99.2 ± 0.37
Cyhalothrin	0.01	78.7 ± 0.86	78.7 ± 0.54
Cypermethrin	0.01	90.8 ± 1.24	90.9 ± 0.74
Deltamethrin	0.05	97.9 ± 0.68	98.0 ± 0.27
Diazinon	1.0	93.9 ± 1.76	94.0 ± 1.03
Dicofol	0.01	96.0 ± 0.79	96.9 ± 0.88
Dieldrin	0.005	82.3 ± 2.08	82.5 ± 1.39
Dimethoate**	0.05	97.2 ± 1.35	97.3 ± 1.30
op'-DDE	0.005	92.1 ± 0.98	92.4 ± 0.66
op'-DDD	0.005	81.9 ± 0.94	82.1 ± 0.52
op'-DDT	0.005	88.4 ± 0.61	87.8 ± 0.53
pp'-DDT	0.005	92.8 ± 1.04	92.6 ± 0.66
Endosulfan	0.005	94.3 ± 0.72	94.1 ± 0.50
Fenpropathrin	0.01	95.9 ± 0.66	95.7 ± 0.44
Fenvalerate	0.05	86.8 ± 1.07	86.6 ± 0.90
Flucythrinate	0.05	96.5 ± 1.66	96.3 ± 1.46
Fluvalinate	0.05	97.1 ± 1.55	96.9 ± 0.38
Heptachlor	0.001	91.7 ± 1.55	91.6 ± 1.71
Methamidophos**	0.05	95.9 ± 1.36	95.5 ± 0.82
Monocrotophos**	1.0	98.1 ± 0.58	98.2 ± 0.18
Parathionmethyl	0.05	90.3 ± 1.29	89.7 ± 1.12
Permethrin	0.01	92.2 ± 1.03	92.1 ± 0.91
Pirimipohosmethyl**	0.005	97.7 ± 0.79	97.7 ± 0.29
Profenofos	0.005	86.7 ± 0.90	86.7 ± 0.66

* Mean of three replicates. ** Analyzed using TSD, other analyses with ECD.

Gas chromatographic analysis. A Varian AG Gas Chromatograph (Model 3600) that was equipped with ^{63}Ni electron capture (ECD) and thermionic specific (TSD) detectors, was used to identify and quantify studied compounds. The equipment was used with a data system DS-650 series (Model DS-651) and a thinkjet printer (Hewlet-Packard).

Two different GC column materials were used to confirm the identity of samples analyzed in this study. Two glass columns each 2 meters long x 2 mm i.d. packed separately with (i) a mixture of 1.5% OV-17+1.95% OV-210 on 80/100 mesh chromosorb W-HP, and (ii) 3% OV-17 supported on 80/100 mesh Chromosorb W-HP were used for the ECD and TSD. The operating parameters for the ECD were: temperatures column oven 230°C, injector 250°C, detector 280°C; attenuation 32, range 10; gas flow: nitrogen (Carrier) 30 ml/min.

The operating parameters for TSD were : temperatures; column oven 200°C, injector 225°C, detector 250°C, attenuation 32, range 12, bead current 3.2 amp, gas flows: nitrogen (Carrier) 30 ml/min, hydrogen 5.4 ml/min and air 175 ml/min.

Each column was conditioned for 24 hr under a slow stream of nitrogen at a temperature slightly higher than the

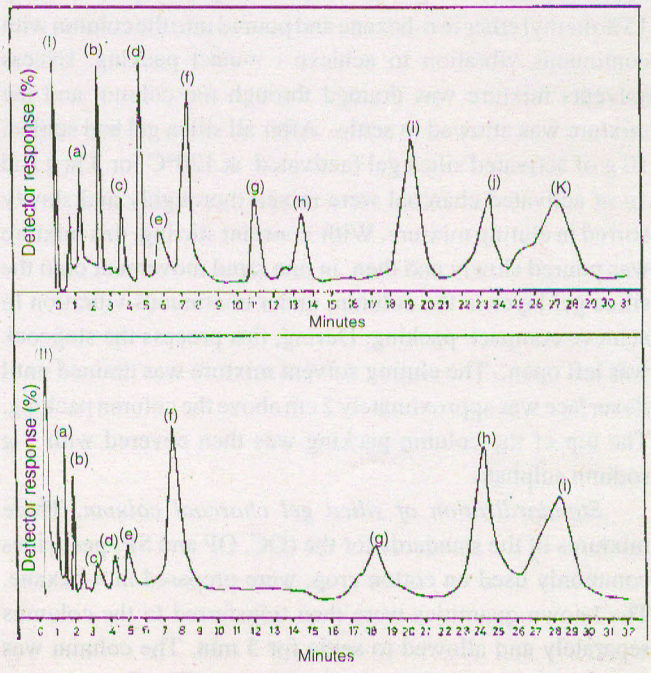

Fig. 1. Gas chromatograms of fortified cotton seeds using 3% OV-17 as stationary phase 80/100 mesh chromosorb W-HP and ECD.

I (a). Pirimiphos-methyl (0.005 ppm), (b) Aldrin (0.001ppm), (c) Dieldrin (0.005ppm), (d) profenofos (0.005ppm), (e) Dicofol (0.01ppm), (f) Bifenthrin (0.01ppm), (g) Fenpropathrin (0.01ppm) (h) Cyhalothrin (0.01ppm), (i) Flualinate (0.05ppm), (j) Cyfluthrin (1.00ppm), and k), Cypermethrin (0.01ppm).

II (a)τ-BHC(0.001ppm), (b) parathion-methyl (0.05ppm), (c)op'-DDE (0.005ppm), (d) op'-DDD (0.005ppm), (e) op'-DDT (0.005ppm), (f) pp'-DDT (0.005ppm), (g)Permethrin (0.01ppm), (h) Flucythrinate (0.05ppm), and (i) Deltamethrin (0.05ppm).

temperature required for working. Similar operating parameters were employed on both the detectors for the two described columns.

Each cleanedup sample extract was gas chromatographed thrice along with its pesticide standard in *n*-hexane using 0.2-2 µl injection. The amount of pesticide in each sample extract was calculated by external standard method.

Results and Discussion

The GC operating parameters used were suitable for quantifying residues of all investigated pesticides. The linearity of response was confirmed by injecting different concentrations of analytical grade pesticides into the GC column and noting their peaks. Retention times for studied compounds on two different detectors were determined (Table 1 and 2). A control sample processed similar to the fortified sample did not show peaks that could be attributed to any studied pesticide. Chromatograms of the pesticides analyzed with the TSD and ECD detectors can be seen in Figs 1-3. The recovery of each pesticide was checked at several ppm (mg kg^{-1}) level but only the lowest limits of determined values (± SE) are given, (Table 3).

In this study, the analytical parameters provided a recovery of OC, OP and SP pesticide residues from fortified and control samples of cotton seeds while maximizing lipid removal. The extraction mixture of acetonitrile with an increasing water content from 0 to 20% improved residues recovery. The solvent mixture of 20% water in acetonitrile not only gave complete recovery of the studied pesticides and their metabolites but also was not soluble to most lipids and undesirable polar coextractives. The alumina basic 90 active Merck Art No. 1076 appeared to be appropriate for the present studies.

For a clear filtration, a comparison was made between filtration with suction and by gravity flow with regard to the amount of lipid material retained by the alumina. Nearly 50% more fat was retained on the alumina when the extraction solvent was filtered by gravity flow.

The all Florisil columns are generally less suitable for cleanup of polar organophosphorus pesticides. Therefore, after partitioning, activated silica gel + charcoal column cleanup was employed to remove the remaining fat and coextractives

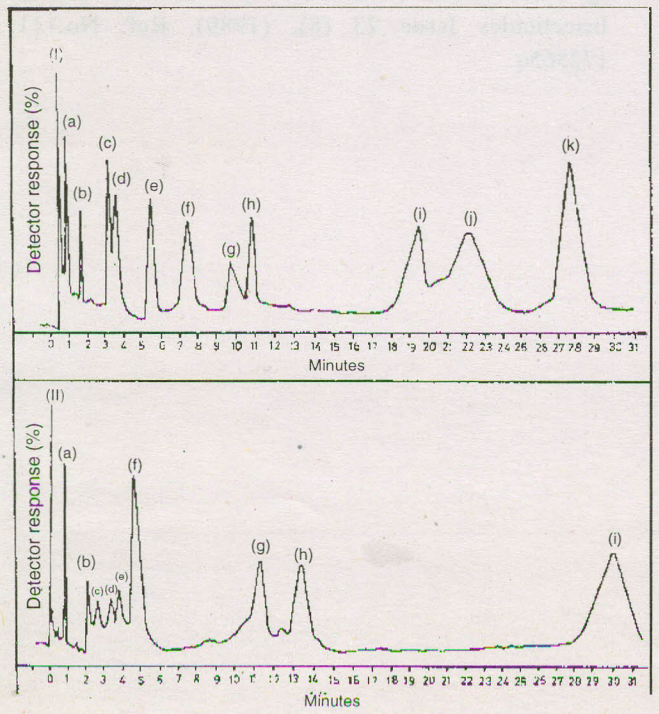

Fig. 2. Gas chromatograms of fortified cotton seeds using a mixture of stationary phase on 80/100 mesh chromosorb W-HP, and ECD.

I (a) Primiphos-methyl (0.005ppm), (b) Aldrin (0.001ppm), (c) Dieldrin (0.005ppm), (d) Profenofos (0.005ppm), (e) Bifenthrin (0.001ppm), (f) Fenpropathrin (0.01ppm), (g) Dicofol (0.01ppm), (h) Cyhalothrin (0.001ppm), (i) Cyfluthrin (1.00.5ppm), (j) Cypermethrin (0.01ppm), and (k) Fluvalinate (0.oppm),

II (a) τ-BHC (0.001ppm), (b) Parathion-methyl (0. 05ppm), (c) op'-DDE (0.005ppm), (d) op'-DDD (0.005ppm), (e) op'-DDT (0.005ppm), (f) pp'-DDT (0.005ppm), (g) Flucythrinate (0.05ppm), (h) Permethrin (0.01ppm), and (i) Deltamethrin (0.05ppm).

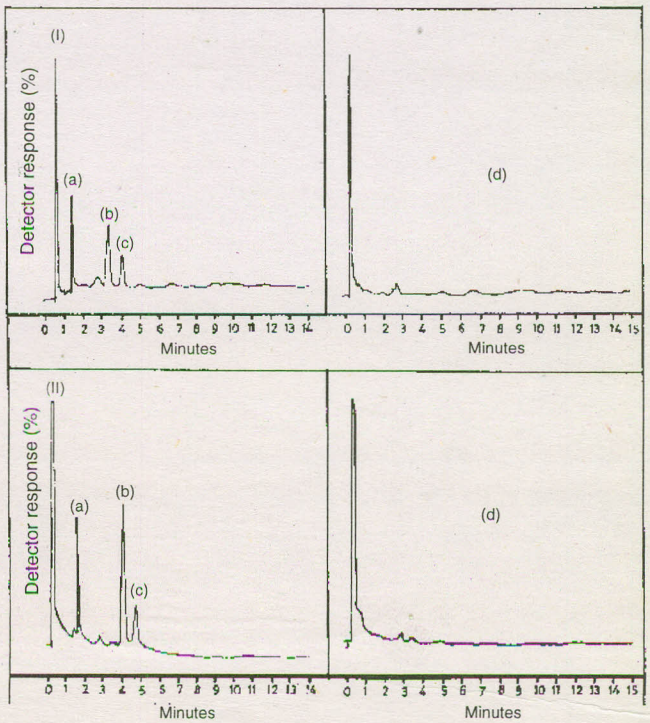

Fig. 3. Gas chromatograms of fortified cotton seeds on TSD using.

I. A mixture of 15% OV-17 + 1.95% OV-210 as stationary phase on 80/100 mesh chromosorb W-HP. (a) Methamidophos (0.05ppm), (b) Monochrotophos (1.0ppm), (c) Dimethoate (0.05ppm) and (d) Control sample.

II. 3% OV-17 as stationary phase on 80/100 mesh chromosorb W-HP. (a) Methamedophos (0.05ppm), (b) Monochrotophos (1.0 ppm), (c) Dimethoate (0.05ppm) and (d) Control sample.

which adversely affect the performance of the electron capture detector. Cleanup by activated silica gel and charcoal and elution by 15% diethyl ether/*n*-hexane produced extracts that contained no extracted fat.

Pesticide residues were determined by electron capture/ thermionic specific gas chromatography using two GC column materials as described in materials and methods. Columns of lower stationary phase loading (6% or less) can usually be operated at such parameters that they will produce maximum efficiency. To vary the retention times of studied pesticides to confirm their identity. Under similar parameters, the residues of 10 OC, 8 OP and 10 SP pesticides and metabolites ranging from 0.001 to 1.0 ppm were successfully identified/quantified using the two column materials (Table-3).

In conclusion, the methodology described in this study is quite sensitive, efficient and suitable for determining multiple pesticide residues (OC, OP and SP) in cotton seeds.

References

1. M. A. Klisenko and Z. F. Yurkova, Vop. Pitan, **26** (6), 68 (1967), From C.A., **68,** 1422 (1969), Ref. No. 76993a, 12345.
2. Jay C. Maitlen, Mc Donough, M. Leslie and Beroza, Morton J. Assoc. Off. Anal. Chem., **52,** (4), 786 (1969).
3. Ralph G. Nash and M. L. Beall, Agron. J. **62,** (3), 365 (1970), From C.A., **73** 230 (1970), Ref. No. 34124r.
4. Rachels Greenberg, J. Agric. Fd. Chem., **29** (4), 856 (1981).
5. L. P. Mildred and A. B. Jerry, J. Assoc. Off. Anal. Chem., **56** (3), 733 (1973).
6. A. L. Milton and M. D. Gregory, Bull. Environ. Contam. Toxicol., **32,** 651 (1984).
7. R. C. Robert and D.I. Roderick, J. Assoc. Off. Anal. Chem., **57** (2), 399 (1974).
8. G. M. Telling, D.J. Sissons and H. W. Brinkman, J. Chromatogr. **137,** 405 (1977).
9. M. L. Hopper, J. Agric. Fd. Chem., **30,** (6), 1038 (1982).
10. J. J. Blaha and P.J. Jackson, J. Assoc. Off. Anal. Chem., **68** (6), 1095 (1985).
11. Claudio Lunardini and Valter Passini, Boll. Chim. Ig. Parte Sci., **40** (52), 65 (1989) From C. A. Selects-Insecticides Issue 23 (8), (1989), Ref. No.111: 172565q .