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Multi-residue Determination of Organophosphorus Pesticides and Synthetic Pyrethroids in Wheat

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ABSTRACT

A multi-residue method for determination of some organophosporus pesticides and synthetic pyrethoids in wheat grain has been developed. Pesticides such as malathion, chlorpyriphos, bifenthrin and permethrin were used for this purpose. After extraction with acetone-methanol (1:1), the extract was partitioned with dichloromethane-sodium sulphate solution. The organic extracts were concentrated and cleaned-up on a glass column containing a mixture of acidic aluminium oxide and activated charcoal (12:1) with eluting solvent dichloromethane. Gas chromatography with an electron capture detector (ECD) was used as analytical tool for quantitative determinations. Known amounts of mixture of pesticides were added to grain extracts prior to extraction, cleanup and GC determinations. Recoveries were checked for two fortification levels; 0.1 and 0.5 mg kg^{-1}. Organophosphorus pesticides were recovered in the range of 68 - 98% and 89 - 102% for 0.1 and 0.5 mg kg^{-1}, respectively. Synthetic pyrethroids were recovered in the range of 70 - 92% and 88 - 98% for 0.1 and 0.5 mg kg^{-1}, respectively.

Key Words: Organophosporus pesticides; Synthetic pyrethoids; ECD

INTRODUCTION

Wheat is the most important cereal crop and constitutes the main component of millions of heads world over. It is frequently stored for long periods of time with consequent risk of heavy insect infestation. Storage in transit is also a critical stage and must be given full attention. It has therefore, become a normal agricultural practice to spray or fumigate stored grains, particularly prior to shipment with contact insecticides so that they reach the port of destination free from any pest infestation. A large number of pesticides are in common use as grain protectants (Daglish *et al*., 2003; Nayak & Daglish, 2006). This usage has been comprehensively reviewed by Haritos (2001). Some pesticides, such as malathion, have been used world wide for over 30 years, whereas others such as bioresmethrin, bromophos, dichlorovos, fenitrothion and pirimiphos-methyl, have been in use for 10 - 22 years. The joint meeting of panel about pesticide residues in food and environment has clearly emphasized this (Arthur, 1992; FAO/WHO, 2002). Soils often receive different fungicides and effect of these has been studied on wheat crop (Fatiha *et al.*, 2006). There are two principal sources of pesticides in wheat grain, firstly, spraying pesticide to growing crops (Jamil *et al.*, 2005; Iqbal & Ali, 2006). Secondly, the admixture of pesticides is used on stored commodities (Wallbank, 1996). But the main route of this contamination is grain protectants. Cereal grains are treated with degradable pesticides, including organophosphate pesticides, carbamates, synthetic pyrethroids and insect growth regulators to prevent insect infestation during storage period (Noble *et al.*, 1982). Cereal grain might be contaminated with several pesticides, which can enter in food chain of human consumption with its consequential hazard. Therefore, it is deemed absolutely necessary to establish several reliable, rapid, inexpensive and effective analytical methods for simultaneous determination of the residues of many pesticides (Fishwick, 1985). A method has been reported to screen most of the commonly used pyrethroid and organophosphorus pesticides in grain by Bottomley and Baker (1984) using GLC and HPLC. A multi-residue method has also been established for fruits/vegetables and wheat grain (Podhorniak *et al.*, 2004).

A rapid screening method using gas chromatography for the determination of resmethrin in wheat flour has been studied by Brown *et al.* (1974). Toteja *et al.* (2006) determined residues of DDT and HCH pesticides in wheat grain and flour samples. A new multi-residue method for simultaneous determination of 405 pesticide residues in grain by accelerated solvent extraction has been developed in which GCMS and LCMS techniques used for quantitative determinations (Guo-Fang *et al.*, 2006).

The selected pesticides in this study are being used to a limited extent in Pakistan for grain protection, and technical data is not available on them. In order to achieve this goal for proper recommendations, knowledge of these aspects is very important. The present study describe extraction, cleanup and gas chromatographic determination of a mixture of two synthetic pyrethroids and two organophosphorus pesticides using gas chromatography (GC) with electron capture detector (ECD).

MATERIALS AND METHODS

Methanol, acetone, dicloromethane, n-hexane and anhydrous sodium sulphate, aluminium oxide pH 4.5, activity 1 and charcoal were used of pesticide analysis grade and purchased from Merck. Anhydrous sodium sulphate was dried at 120°C aluminium oxide activated at 450°C for three hours and cotton wool was washed with a mixture of acetone and hexane (1 + 1) prior to analysis. Filter papers of Whatman # 542 and chromatographic glass column of 450 mm length and 25 mm id were used for analysis. Centrifuge apparatus (Dynac), rotary evaporator with chiller (Buchi, Model V-512), gas chromatograph (Agilent technologies 6890 N) equipped with an auto injector (7683 series) were used. Ni^{63} electron capture detector (ECD), Fused silica capillary column with length of 30 m and 0.25 mm id (HP-5 MS) with film thickness 0.25 um and nitrogen as carrier gas were selected for gas chromatography (GC). A 5 g sample of grounded wheat grain was transferred into a conical flask and 50 mL of acetone-methanol (1 + 1) extraction solvent was added into it. The content of the flask were shaken for 3 h continuously on mechanical flask shaker. Then the solvent extracted material was filtered in measuring cylinder and volume of extract was noted down. The extract was then transferred to a separatory funnel containing 200 mL of sodium sulphate solution (2.5 g per 100 mL) and 25 mL of dichloromethane, then shaken it vigorously for 2 min. The phases were allowed to separate and collect the lower layer of dichloromethane into a 250 mL conical flask. Partitioning of aqueous layer was repeated twice with two 25 mL portions of dichloromethane and the combined dichloromethane extracts passed through 25 g of anhydrous sodium sulphate in a 450 mm x 25 mm id glass column then dry extract was collected in a conical flask. Finally, the sodium sulphate column was washed with 10 mL of dichloromethane and then concentrated the combined extracts and washing to about 1ml on a rotary evaporator. The aqueous extracts were discarded. For column clean-up 1 mL dichloromethane extract was transferred on to a 450 mm x 25 mm id glass column containing 15 g mixture of aluminium oxide and charcoal (12:1) that slurry-packed with dichloromethane. The solution was allowed to pass through the column until the liquid level reached the top of the column. Rotary flask was rinsed with two 5 mL portions of dichloromethane, transferred the washing to the column and eluted the column with dichloromethane. Flow rate was adjusted to 1 mL/min^{-1} and 120 mL eluate was collected. Combined washings and eluate were concentrated to dryness on a rotary evaporator and dissolved the residue in 1 mL hexane. Then injected 1 µL of dissolved residues to gas chromatograph through auto injector and peak areas were compared with those obtained from similar injections of standards. The samples were left for 30 min after addition of the pesticides, so that the pesticides were thoroughly absorbed, before proceeding to extraction, cleanup and determination in accordance with method described above. One blank and one control were also run in the same manner. Each analysis was performed in triplicate. Gas chromatographic parameters are presented in Table I.

Table I. Gas chromatographic parameters for studied pesticide

Column (oven)	260°C
Flow of carrier gas (N_2)	$0.8 ml min^{-1}$
Mode	Splitless
Injector	280°C
Injection volume	1µl
Purge flow	$20 ml min^{-1}$
Purge time	1min
Detector	300°C
Make-up gas flow	$60 ml min^{-1}$

RESULTS AND DISCUSSION

For selection of extracting mixture and eluting solvent, the method of Bottomley and Baker (1984) was followed, whereas the rest of method was completely developed in our laboratory. Percent recoveries of four pesticides from fortified wheat grain extracts are presented in Table II. Satisfactory results were obtained for all four pesticides at two fortification levels, which are 0.1 mg kg^{-1} and 0.5 mg kg^{-1} mg/kg levels. Organophosphorus pesticides are recovered in the range of 68 - 98% and 89 - 102% for 0.1 and 0.5 mg kg^{-1}, respectively. Synthetic pyrethroids are recovered in the range of 70 - 92% and 88 - 98% for 0.1 and 0.5 mg kg^{-1}, respectively. These results show the method has suitable range with good reproducibility. Mininmum residue limits (MRLs) recommended by WHO/FAO (Greene & Pohanish, 2005) for malathion, chlorpyriphos, bifenthrin and

Table II. MRLs, limits of determination, fortification levels, percent recoveries and percent RSD for studied pesticide

Pesticides	Maximum residue limit FAO/WHO ($mgkg^{-1}$)	Limit of detection ($mgkg^{-1}$)	Retention Time (min)	Fortification level ($mgkg^{-1}$)	Recoveries (%)
Bifenthrin	1.0	0.01	10.68	0.1	91, 92, 90
				0.5	96, 98, 98
Permethrin	2.0	0.05	13.65+13.93	0.1	70, 72, 72
			(both isomers)	0.5	88, 89, 88
Chlorpyriphos	1.0	0.05	6.48	0.1	70, 68, 70
				0.5	89, 90, 90
Malation	8.0	0.05	5.82	0.1	98, 97, 98
				0.5	100, 101, 102

Fig. 1. Blank solvent quantifying on a 30 m HP-5 MS capillary column showing some non-interfering peaks

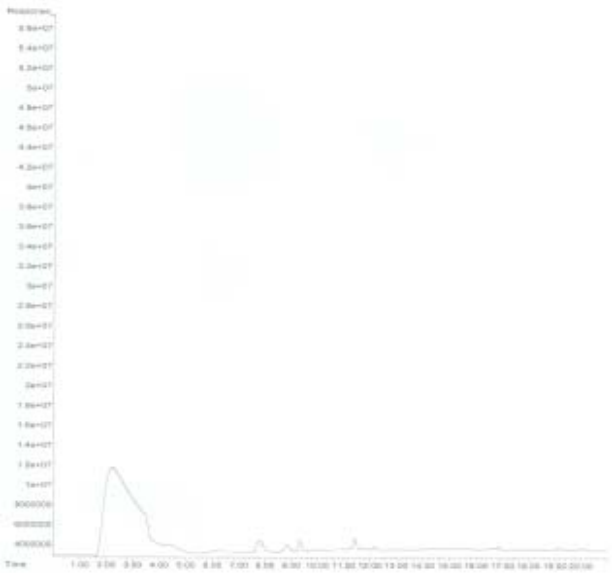
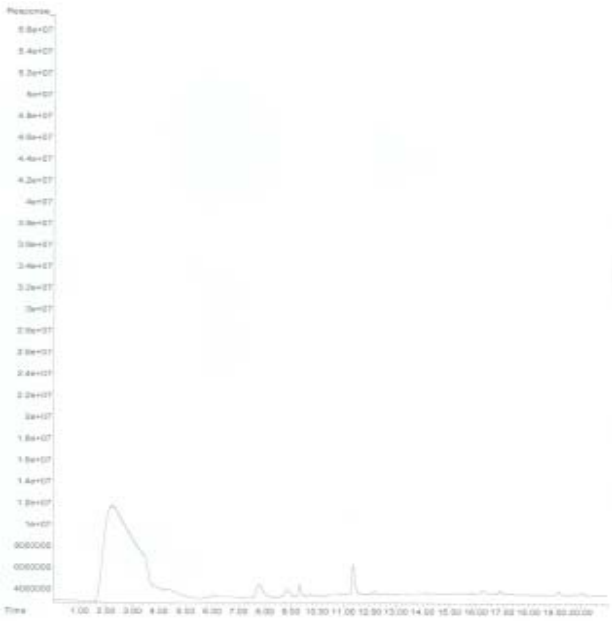


Fig. 2. Control sample quantifying on a 30 m HP-5 MS capillary column showing some non-interfering peaks



permethrin, respecively in cereal grains are listed in Table II. According to WHO/FAO, the residue analytical methods should be so sensitive that at least the lower permissible levels can be quantitatively determined. The forgoing results are in full agreement with this recommendation. The results are comparatively better than previously obtained by Bottomley and Baker (1984), who worked on multi-residue determination of synthetic pyrethroids and organophosphorus pesticides in grain by GLC and HPLC. Generally, organophosphorus pesticides are determined

Fig. 3. Separation of four pesticides (0.5 ng of each) quantifying on a 30 m HP-5MS capillary column, Peaks: 1. malathion, 2. chlorpyriphos, 3. bifenthrin and 4. permethrin (a, b)

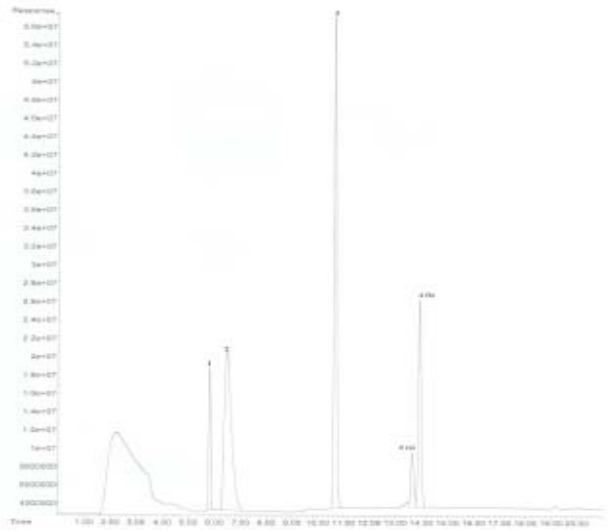
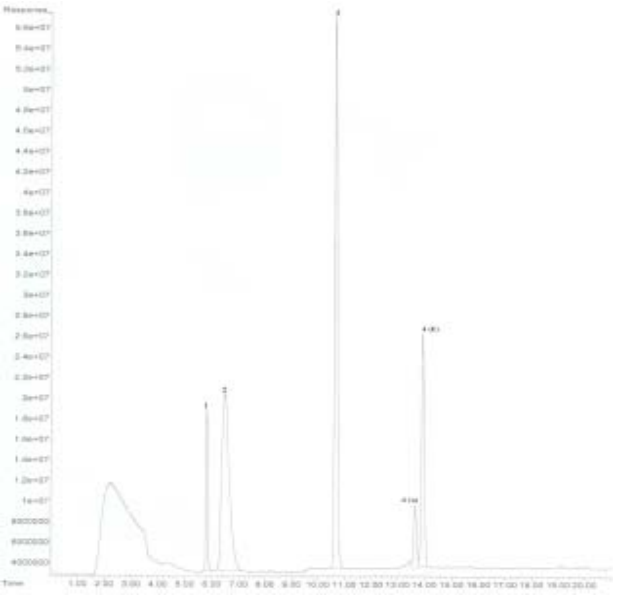


Fig. 4. Separation of four pesticides in wheat grain fortified at 0.1 mg/kg quantifying on a 30 m HP-5 MS capillary column, Peaks: 1. malathion, 2. chlorpyriphos, 3. bifenthrin and 4. permethrin (a, b)



quantitatively by GC using flame ionization detectors or flame photometric detectors Bottomley and Baker (1984), whereas in the present study only electron capture detector has been used. So, the synthetic pyrethroids as well as organophosphorus pesticides have been studied at a time with controlled parameters of instrument. The GC operating parameters used were suitable for quantifying residues of all investigated pesticides. The linearity response was

confirmed by injecting different concentrations of analytical grade pesticides into the GC column and noting their peaks. Retention times for studied pesticides were determined (Table II). A blank and control sample processed similar to the fortified sample did not show peaks that could be attributed to any studied pesticide (Fig. 1 & 2). In blank, all steps of method were applied only to the solvent system. In control, the grain samples without addition of pesticides passed through all steps. The purpose of blank and control used was to check accuracy of the method. The cleanup requirements differed from sample to sample or presence of specific physicochemical properties to separate the desired compounds from the sample extractives. Insufficient cleanup of sample causes rapid deterioration of gas chromatographic system thereby precluding reliable results (Zahida *et al*., 1994). Elution of mixture of analytical standards of pesticides is presented in Fig. 3 and 4 illustrated the elution of pesticides from fortified samples at the operating conditions already described. Single sharp peaks and smooth base line were observed in all cases, which prove that sample is cleaned up perfectly and free from undesired coextractives.

We have developed an easy and inexpensive multi-residue method for determination of pesticides in grain, commonly used in Pakistan. The method is efficient and reliable and allowed lipid removal to a large extent. The method is simple, efficient and sensitive. It is expected that the above described procedure would also prove suitable for other organophosphorus pesticides and synthetic pyrethroids.

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