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Multi-residue Determination of Synthetic Pyrethroids and Organophosphorus Pesticides in Whole Wheat Flour using Gas Chromatography

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Wheat grain in the form of flour and its processed products have become and essential part of human diet. The consumption of wheat flour and its products is increasing everyday throughout the world. As a result of increasing demand in Pakistan, cultivation and production of wheat crop has increased tremendously. However, the system still in primitive and is facing many pre and post harvest problems that requiring to be addressed. There are two principal sources of pesticides contamination in stored commodities, pesticide residues resulting from field spraying and the residues accumulated as a result of pesticides' admixture with grain or fabric treatment during storage. Cereal grain are treated with degradable pesticides, including organophosphates, carbamates, synthetic pyrethroids and insect growth regulators in storage premises as well as prior to shipment to other countries to prevent insect infestation. In this regard, residual efficacy of some pesticides in stored wheat studied by Arthur (1992). Noble et al. (1982) studied the stability of pyrethroids. A large number of pesticides are in common use as grain protectants. This usage has been comprehensively reviewed by Snelson (1987). The identification and quantification of pesticide residues in flour as well as in its products is important because small quantity of pesticides or their metabolites may persist in flour after processing or refining even in baked or cooked products (Skerritt et al. 1996). Therefore it is necessary to monitor flour for pesticides residues.

A collaborative study on method development of organophosphorus pesticide residues in wheat has been reported (Desmarchelier et al. 1977). Bottomley and Baker (1984) developed a method to screen most of the commonly used pyrethroid and organophosphorus pesticides in grain using gas chromatography. Fishwick (1985) studied a wide range of organophosphorus pesticides residues in grain. Brown et al. (1974) reported a rapid screening method using gas chromatography for the determination of resmethrin in wheat flour. A multi-residue method for *N*-methyl carbamates and metabolite pesticide residues at ppt level for fruits/vegetables and wheat grain was reported by Podhorniak et al. (2004). A new multi-residue method for simultaneous determination of 405 pesticide residues in grain has also been developed (Guo-Fang et al. 2006). Toteja et al. (2006) determined residues of DDT and HCH pesticides in wheat grain and flour samples.

The present study was restricted to pesticides which are internationally used as grain protectants. These pesticides are also used in Pakistan, however the technical data relating to the contamination in finished products is not available. Periodical monitoring of grain and flour at various supply channels for multiple pesticide residues is therefore desirable in order to protect the consumer from the possible health hazards. In the present study samples of whole wheat flour were screened for residues of a mixture of five synthetic pyrethroids and six organophosphorus pesticides using gas chromatography with electron capture

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Table 1 MRLs, Detection limits, fortification levels, percent recoveries and percent RSD for studied pesticides

Studied pesticides	FAO/WHOMRLs (μg/g)	Detection limit (μg/g)	Fortification level (μg/g)	Recoveries (%)	RSD (%)
Bifenthrin	1.0	0.01	0.1 0.5	90, 89, 92 99, 101, 100	1.69 1.00
Lambda-Cyhalothrin	0.5	0.01	0.1 0.5	74, 70, 75 89, 90, 95	3.62 3.51
Deltamethrin	2.0	0.05	0.1 0.5	74, 72, 70 95, 97, 95	1.93 1.20
Permethrin	2.0	0.05	0.1 0.5	85, 90, 80 99, 101, 102	5.88 1.51
Cypermethrin	2.0	0.05	0.1 0.5	99, 100, 93 101,105,100	3.88 2.59
Chlorpyriphos	1.0	0.05	0.1 0.5	90, 92, 91 112, 113, 99	1.09 7.23
Fenitrothion	10.0	0.01	0.1 0.5	79, 87, 87 95, 99, 98	5.47 2.13
Malation	8.0	0.05	0.1 0.5	98, 91, 92 100, 101, 99	4.04 1.00
Chlorpyriphos-methyl	10.0	0.01	0.1 0.5	78, 79, 80 90, 92, 95	1.26 2.72

detector. The objective of this study is to develop a rapid procedure for analysis of multi-residue pesticides in wheat flour for routine monitoring.

Materials and Methods

Methanol, acetone, dichloromethane, n-hexane and anhydrous sodium sulphate and activated graphitized charcoal were of analytical reagent grade and were from Merck (Darmstadt, Germany). Aluminium oxide pH 4.5 ± 0.5, activity 1 was of analytical reagent grade purchased from Faluka Laboratory Supplies (Switzerland). Anhydrous sodium sulphate was dried at 120°C while aluminium oxide activated at 450°C for 3 h. Cotton wool washed with a mixture of acetone and hexane (1 + 1) prior to analysis. Pesticides standards were purchased from Riedel–de Haën, Siga-aldrich laborchemikalien GbH (Germany). Stock standard solutions of the pesticides were prepared in *n*-hexane (synthetic pyrethroids) or acetone (organophosphorus pesticides). Working standard solutions were prepared as per requirement from the pesticide stock solution in hexane or acetone. These solutions were all stable for at least 1 month if stored in the dark at 4°C.

A gas chromatograph (Agilent technologies 6890 N, USA), equipped with an auto injector (7683 series) and Ni^{63} electron capture detector, for data collection a software enhanced data analysis was present in the system. The laser-jet printer (Hewlet Packard 2300) and capillary column of 30 m × 0.25 mm id (HP-5MS) with film thickness 0.25 μm was used. Different temperature programs were arranged for synthetic pyrethroids and organophosphorus pesticides. In temperature program(1) the parameters were set for the synthetic pyrethroids in which injector temperature was 280°C and the column oven temperature was 260°C with a nitrogen flow rate of 0.8 mL min^{-1}. While the temperature program(2) was set for organophosphorus pesticides in which injector temperature was 240°C and column oven temperature was 190°C with a nitrogen flow rate of 0.5 mL min^{-1}, temperature for detector was kept constant at 300°C in both cases.

Wheat grain of 1 kg was drawn from a local farmer and made flour in a laboratory mill. Whole wheat flour treated as control was fortified with known quantities of each studied pesticides separately as well as in a mixture and was allowed to stand for 3 h so that the pesticides were thoroughly absorbed. These samples along with a blank (only solvent passed through all the steps) as well as control sample (the sample free from any pesticides) were then processed through the following procedure in triplicate and finally analyzed for percent recovery by gas chromatograph.

A whole wheat flour of 5 g was transferred into a 100 mL centrifuge tube and added to it 50 mL of acetone–methanol (1 + 1) extraction mixture. The contents of the tube were homogenized by constant stirring with a glass-rod for 3 min. The contents were centrifuged at 2,500 rpm min^{-1} for 3 min and then directly decanted the supernatant liquid into a 100 mL cylinder. Further 40 mL of acetone–methanol (1 + 1) extraction mixture was added to the centrifuge tube and homogenized with a glass-rod for 3 min, again centrifuged the it for 3 min and decanted in the same cylinder. The extract was then transferred to a 1 L separatory funnel. About 200 mL of sodium sulphate solution (2.5 g per 100 mL) was added to the separatory funnel, followed by 25 mL of dichloromethane and shaken 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. The combined dichloromethane extracts was passed through 25 g of anhydrous sodium sulphate in a 450 mm × 25 mm id glass column. Dry extract was collected in a conical flask and finally, the sodium sulphate column was washed with 10 mL of dichloromethane. Then concentrated the combined extracts and washing to about

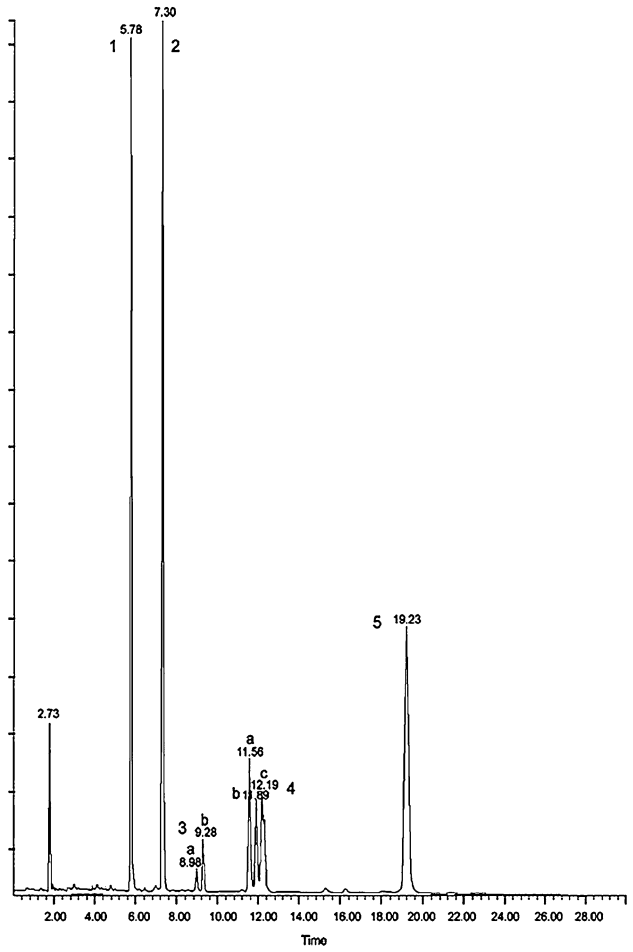

Fig. 1 Separation of five synthetic pyrethroids (0.4 ng of each) on a 30 m HP-5MS capillary column, temperature program. *1* Bifentherin, *2* lambda-cyhalothrin, *3* (a, b) isomers of permithrin, *4* (a, b, c) isomers of cypermethrin and *5* deltamethrin

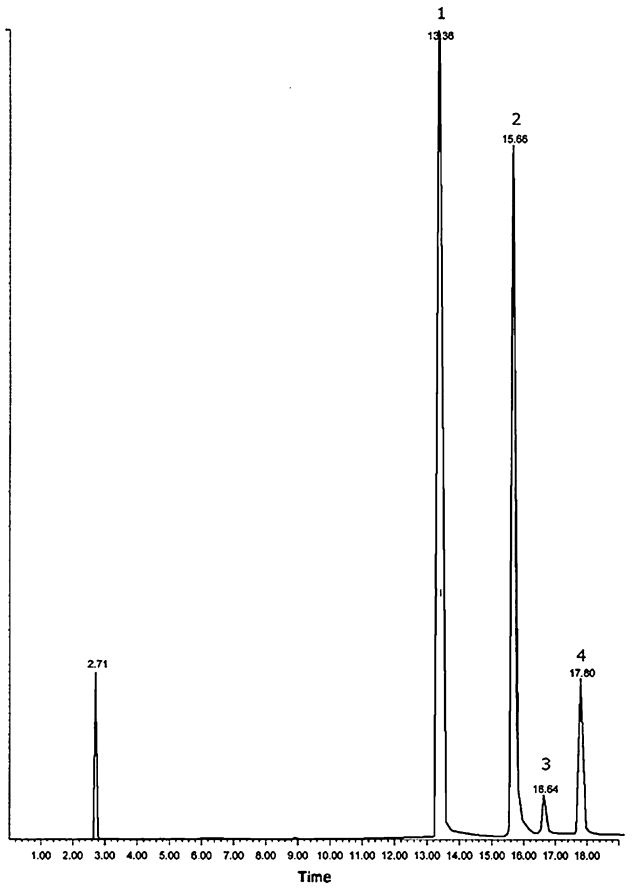

Fig. 2 Separation of four organophosphorus pesticides (0.4 ng of each) on a 30 m HP-5MS capillary column, temperature program. *1* Chlorpyriphos-methyl, *2* fenitrothion, *3* malathion and *4* chlorpyriphos

1 mL on rotary evaporator. The aqueous extracts were discarded.

For column clean-up 1 mL dichloromethane extract was transferred on to a 450 mm × 25 mm id glass column containing 15 g aluminium oxide that has been slurry-packed with dichloromethane. The solution was allowed to pass through the column until the liquid level reached the top of the column, then eluted with dichloromethane. Flow rate was adjusted to 1 mL/min^{-1} and 200 mL eluate was collected. Combined washings and eluate were concentrated to 1 mL on rotary evaporator.

For further cleanup a polypropylene mini column was plugged with cotton wool, packed with about 4 g mixture of aluminium oxide and activated charcoal (12 + 1) in between two layers of 1 gm each of anhydrous sodium sulphate. The column has been pre-washed with 10 mL dichlromethane and the concentrated extract was then transferred on to a polypropylene mini column. A total of 15 mL eluate was collected and evaporated to dryness. The residues were dissolved in 1 mL hexane or acetone. From the dissolved residues, 1 μL was injected to gas chromatograph through auto injector and peaks areas were compared with those obtained from similar injections of standards.

Results and Discussion

For extraction and clean up, method of Bottomley and Baker (1984) was followed in which they worked on wheat grain whereas in present study wheat flour was used. The cleanup step was little bit modified with addition of propylene mini column to remove the remaining fat and co-extractives in the extract because insufficient cleanup of sample causes rapid deterioration of gas chromatographic system especially electron capture detector, thereby precluding reliable results. For the determination of residues, electron capture detector was used with two different temperature programs for organophosphorus and synthetic pyrethroids to get good separation. Blank as well as control sample did not show any peak that could be attributed to

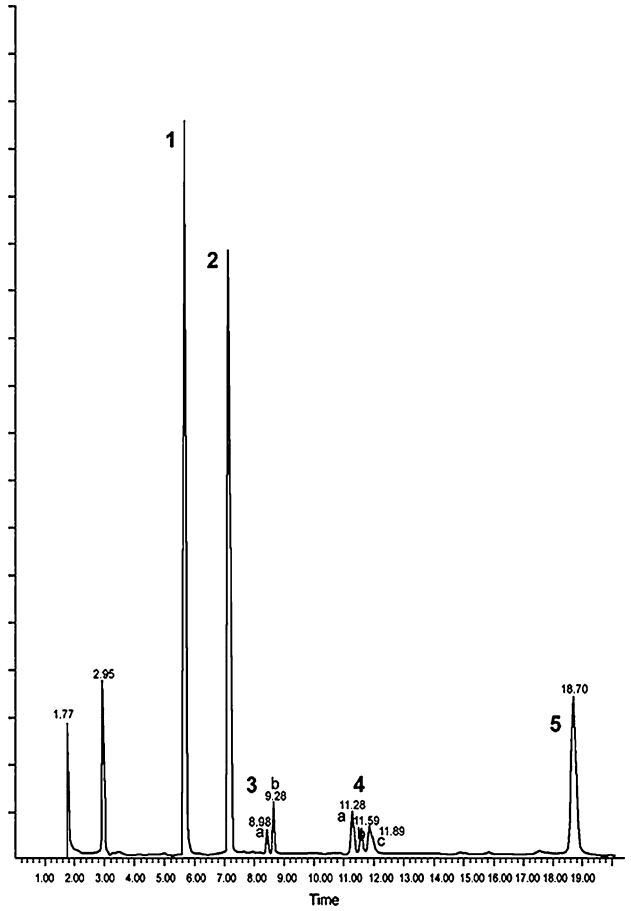

Fig. 3 Separation of five synthetic pyrethroids in wheat flour fortified at 0.1 μg/g on a 30 m HP—5 MS capillary column. *1* Bifenthrin; *2* lambda-cyhalothrin; *3* (a, b) isomers of permethrin; *4* (a, b, c) isomers of cypermethrin and *5* deltamethrin

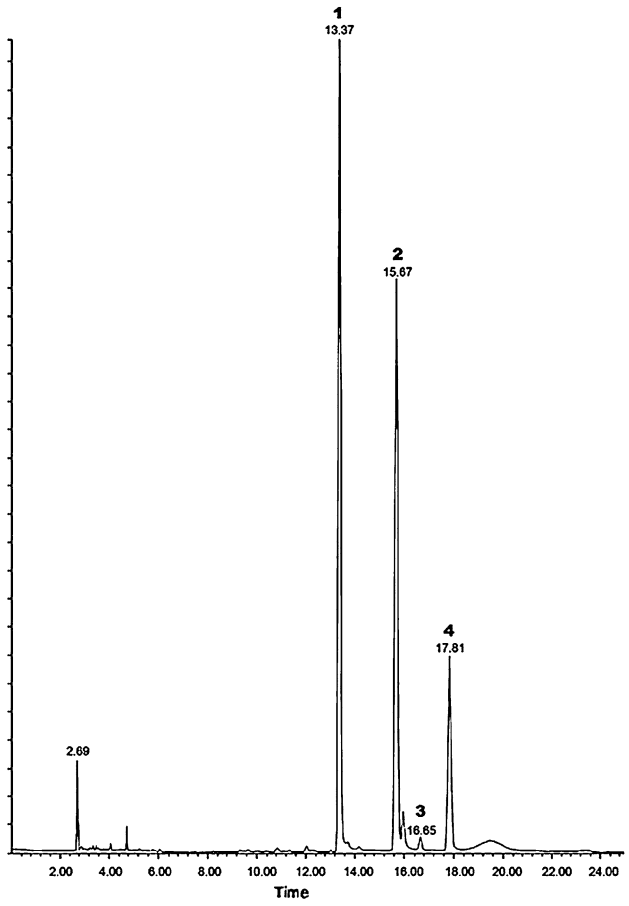

Fig. 4 Separation of four organo phosphorus pesticides in wheat flour fortified at 0.1 μg/g on a 30 m HP-5MS capillary column. *1* Chlorpyrifos-methyl, *2* fenitrothion, *3* melathion and *4* chlorpyrifos

the studied pesticides. Detection limits, which are actually the sensitivity of instrument for studied pesticides and maximum residue limits (MRLs) (Report 2002) are listed in Table 1. Recoveries were performed for two fortification levels are also presented in Table 1 with their RSD% from which it is apparent that the average range of recovery of the pesticides determined by the method fall within 70 and 113% and RSD% was in the range of 1.00–7.23%. These results demonstrate that the method has relatively good reproducibility. The above data prove that the recovery of pesticides is quite satisfactory. It is interesting to note that in all the cases at lower fortification level 0.1 μg/g the percent recovery decreases. It is due to the fact that at lower residue concentrations chances of error are usually enhanced but the reported results are within the range of permissible error. Gas chromatograms of the studied organophosphorus pesticides and synthetic pyrethroids are presented in Figs. 1, and 2, respectively, having 0.4 ng each standard. Gas chromatograms of the fortified samples (0.1 μg/g) are represented in Figs. 3, and 4, respectively.

The present study has resulted in development an easy and inexpensive multi-residue method for determination of pesticides in flour commonly used in Pakistan. This study focused on easy operation. There are so many sophisticated techniques and methods for extraction as well as automated sample cleanup systems that have been worldwide introduced but those are very expensive and difficult to approach. So there is need to develop any economic way to screen the multiple pesticide residues. This method can also be applied to organochlorine pesticides as the method is efficient, reliable and allowed lipid removal to a large extent.

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