

Gas Chromatographic Multi-Residue Pesticide Determination Method for Cereal Grains

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Abstract: A simple, cheap, easy and efficient multi-residue analytical method is reported for simultaneous determination of sixteen different multiple pesticide residues including organochlorines, organophosphates, synthetic pyrethroids and herbicides in rice grains and for the analysis of thirteen insecticide residues in wheat grains. Extraction was carried out by a mixture of acetone and methanol. A mixture containing acidic aluminum oxide and activated charcoal was used for the cleanup using dichloromethane as elution solvent. For instrumental analysis a capillary gas chromatography connected with electron captured detector (GC-ECD) and a capillary column (HP-5MS) was used. Residual analysis of qualitative and quantitative determination was carried out on the basis of retention times and peak area respectively. The method was tested in terms of LODs and LOQs. Precision and recoveries of the method were evaluated at two different concentration levels; 100µg/Kg and 500µg/Kg for each matrix. Mean recoveries (73.77 to 100.170%) with %RSD values between 2.21 and 9.27% for spiked wheat grains samples and 74.0 to 104.67% with %RSD values in the range of 2.41 to 12.42%, for spiked rice samples were obtained. The method was also validated by participating in the FAPAS® proficiency tests (PTs) programs in the year 2010 and 2011, for rice and wheat respectively. Good Z-score values of less than 2 were obtained that proven the reliability of the method. This method was successfully implemented for the pesticide residues analysis of two hundred grain samples of wheat and rice.

Key words: Multiple pesticide residues • Insecticides • Wheat grain • Rice grain • GC-ECD

INTRODUCTION

Cereal grains are the most important food grains because they are the chief source of food for the majority of the world's population. They provide about 60% of the calories and about 50% of the proteins to the human race. Like many other countries the economies of Pakistan depends directly upon cereals and indirectly the prosperity of all nations depends upon increased production of cereals. Wheat and rice are the major cereal crops of the world. Among the food grain crops, wheat occupied the largest crop area and the quantity produced is more than that of any other crop. Wheat crop occupies about 17% of the world's cropped area and its contribution is 35% of the staple food [1]. Flour of wheat grains and their finished commodities are the important and necessary component of our daily diets [2].

Pakistan is contributing 3.72 percent cropping area of the total world wheat area with approximately 3.17 percent grains production and its rank is at 8th position among the top wheat producing countries [3]. In addition to wheat,

rice is one of the leading summer cereal grains of the world. On consumption basis among cereal commodities its rank is 3rd in the whole world. It is considered and included among the top world starchy food items.

Amongst the twelve Asia and Pacific regional countries, Pakistan is the second largest pesticides consumer country and occupies a disadvantageous place in terms of safe use of pesticides because the majority of Pakistani farmers are illiterate and unskilled [4]. Periodical monitoring of cereal grains for multiple pesticide residues is therefore, desirable in order to protect the consumer from the possible health hazards. However, in Pakistan there is no technical published data related to the contamination of pesticide residues in cereal grains except a study reported the residues of Lambda-Cyhalothrin and Malathion in rice samples of Punjab [5]. Dichlorodiphenyltrichloroethane (DDT) was included as one of the twelve persistent organic pollutants in 2004, while α -BHC, β -BHC and Lindane were added to the list on the Stockholm Convention in 2009 [6].

Despite that some countries are using chlorinated pesticides as a number of researchers have reported the contaminations of chlorinated pesticide residues in wheat and rice in their respective countries. A study conducted in India showed the residues of DDT and BHC isomers in wheat samples of Jaipur and Rajasthan [7]. Similarly, researcher also reported different isomers of BHC in about 45-80% of the wheat grains and wheat flour samples of India [8]. Among the BHC isomers, Lindane was founded greater than 0.1mg/kg in five samples of wheat grains out of 1080 samples and in 340 wheat flour samples out of 632 samples. A research study stated that Malathion, chlorpyrifos and endosulfan are widely used on paddy cultivation in India and reported the residues of chlorpyrifos and endosulfan insecticides in rice grains [9]. Ahuja and Awasthi [10] also reported the alarming levels of the residues of α -BHC, β -BHC and Lindane isomers of BHC in different market samples of wheat and rice grains of India. In Serbia, Skrbic and Predojevic founded the residues of endosulfan and BHC isomers, i.e. α -BHC, β -BHC and lindane in wheat flour samples [11]. Another monitoring study conducted in South Korea, reported permethrin, lambda-cyhalothrin and chlorpyrifos residues in rice samples [12]. In Mexico, a study reported five insecticides i.e. cypermethrin, deltamethrin, Malathion, chlorpyrifos and DDT isomers residues in wheat grains samples [13]. Nevertheless, Pakistan import a huge amount of wheat from various other countries, therefore, pesticide polluted consignment could be received as a matter of fact.

The present study is therefore, to focus on method evaluation for the simultaneous determination of multi-group residues of pesticides in cereal grains of rice and wheat grains. Another purpose of the present research study was to present a methodology that can be easily applied by those laboratories that have no approach and facilities to new sophisticated current extraction techniques and equipment like gas chromatography coupled with selective mass detector.

MATERIALS AND METHODS

Chemicals and Reagents: Throughout the experimental process, analytical grade solvents and other chemicals were used. All the chemicals were purchased from Merck (Germany) while aluminum oxide Acidic, pH 4.5 \pm 0.5, Brockmann activity1 was purchases from Fluka (Switzerland).

Preparation of Pesticide Standards Solutions: Each (individual) stock solution of the studied pesticides was prepared in 10ml volumetric flasks by accurately weighed analytical grade pesticide standard dissolved in hexane. Among these stock solutions, a mixture of thirteen insecticides and three herbicides representing a concentration of each pesticide i.e. 1 μ g/ml was prepared in a 50ml volumetric flask. This working standard solution was throughout used for analysis of pesticides in rice. For the analysis of pesticides in wheat grains, another mixture was prepared in 50 ml volumetric flask containing concentrations of each insecticide i.e. 1 μ g/ml.

Fortification: Each sample (appx. 250 g) was ground with a mechanical hand grinder separately. Four grams of each wheat and rice ground samples were then taken in triplicate in 100ml beaker. Calculated volume of each studied pesticide standard solution was separately added for fortification purpose. For complete absorption of the residues, the spiked rice and wheat samples were kept for 24 hours at room temperature before extraction. Along with these spiked samples, a control ground sample of 4gms each of wheat and rice as well as a blank were simultaneously processed for extractions.

Extraction: A mixture of methanol and acetone in the ratio of 1:1 was prepared before starting the extraction procedure. Fortified samples along with a control and a blank were transferred into a 50ml centrifuge tubes, having a screw caps. Forty milliliter of extraction solvents mixture containing methanol and acetone (1:1) was added to the contents of each centrifuge tube. The contents of the tube were thoroughly homogenized by two minutes vigorously hand shaking. All the tubes were centrifuged at a speed of 2500 rpm (round per minute) for 3 minutes at room temperature. The supernatant extraction solvents layer was decanted in to a 300ml conical flask through a filter funnel. Additional 35 ml of extraction solvents mixture was added to the contents of the centrifuge tube followed by vigorously hand shaking for 2 minutes. Again all the centrifuge tubes were centrifuged at the same speed of 2500 rpm for three minutes. The upper solvents layer of the tube was decanted through the filter funnel with a Whatman's filter paper into the same 300ml conical flask.

Clean-up: Acetone-methanol extract was transferred into a 500ml separatory funnel. Added 200ml of 2.5% (w/v) anhydrous Na₂SO₄ solution followed by 25ml of DCM

(dichloromethane) and vigorously hand shaking for 2 minutes. The layers were allowed to separate and the lower layer of dichloromethane was passed through 25g of anhydrous sodium sulphate in a 58cm x 2.4cm i.d. glass column that has been plugged with a cotton wool at its narrow end. Sodium sulphate, before pouring in glass column, was heated in furnace at 500°C for 3 hours. This partitioning of aqueous extract layer was repeated twice and 75mL of extract eluted from column. An excess of 10 of DCM was also passed through column and the final volume was concentrated at 40°C on rotary evaporator and the volume was reduced to approximately 2ml.

A cotton wool was placed at the bottom of chromatographic column (58cm x 2.4cm i.d.). A mixture containing activated charcoal (at 300°C for 3 hrs) and acidic aluminum oxide (at 500°C for 3 hrs) at the ratio of 1:15, was slurry packed with dichloromethane. A layer of 5 gm anhydrous sodium sulphate was placed above and below the alumina/charcoal bed.

After preparation of this glass column, 2mL concentrated sample extract was passed followed by 160mL DCM. Chromatographic glass column valve was adjusted to allow elute to flow at the rate of 1mL/min. Elute was collected into volumetric flask. All the eluate was evaporated completely by rotary evaporator on 40°C temperature. The residues were dissolved in 2ml of n-hexane. These dissolved residues were directly transferred from rotary flask into GC vial.

Instrumental Analysis: All the analysis was performed on GC. The injection mode was splitless, Nitrogen gas (Grade 5) was used as carrier (1mL min⁻¹) and makeup flow 60mL min⁻¹. Inlet and detector temperatures were 280°C and 320°C respectively. However, oven temperature was programmed at 80°C, held for 1min, increased at 30°C min⁻¹ to 180°C, increased 3°C min⁻¹ to 205°C, held for 4min and finally increased at 20°C min⁻¹ to 290°C and held for 5min.

One microlitre aliquot of extracts was injected into the gas chromatography through auto injector. Each sample-extract was injected twice. A mixture of pesticides standard solution of 1 µg/ml was injected prior to injection of the treated samples and again after every sixth injection of treated samples.

Detection Limit (LOD) and Quantification Limit (LOQ): Quantification Limit (LOQ) is the minimum detectable amount of pesticide residue in wheat and rice items with

an acceptable mean recovery (70-110%) along with acceptable relative standard deviation (RSD ≤ 10%). It can be measured with reference to S/N (signal to noise) ratio from the base line.

LOD and LOQ of the studied pesticides were determined by the method of Keith *et al.* [14]. In a fortified sample, concentration of each pesticide giving a peak-to-peak noise ≥ 3 with respect to the baseline, was considered as LOD and the concentration giving a peak-to-peak noise ≥ 10 with respect to the baseline, was considered as LOQ.

Calibration of Instrument: Linearity of the Gas chromatographic (GC) system was checked by analyzing six different concentrations (each of the concentration was injected in duplicate) of four different selected pesticides' standards. The lowest values of each selected pesticide standard concentration were chosen on the basis of their limit of detection (LOD) values. The highest range of pesticides standards concentration was selected within the value at least thirty times of the limit of detection (LOD) of the respective pesticide.

Method Validation: The developed method was validated by three methods. First the recovery of the fortified samples of both rice and wheat. Second method of validation was performed by analyzing commercial samples of wheat and rice received from exporter, importer and domestic store grain samples. Third validation test was performed by participating in the FAPAS proficiency tests for wheat (in 2010) and rice (in 2011).

RESULTS AND DISCUSSION

The detection limits (LODs), limits of quantification (LOQs) and retention times (RTs) of the studied pesticides for rice and wheat are given in Tables 1. In the rice samples, LODs and LOQs were found in the range of 1 to 50µg/kg and 2 to 100µg/kg, respectively, whereas, in the wheat grain samples, these were found to be 1 to 20 µg/kg and 2 to 50µg/kg, respectively. Representative chromatogram of the studied pesticides standards and recoveries of respective pesticides in fortified rice and wheat grains sample obtained at 100µg/kg fortification levels are shown in Fig. 1. The percent recoveries of pesticides in rice and wheat grains are given in Table 2. Results showed that the average recovery of the method for rice grain samples at 100µg/kg fortification level is between 74% and 104.67% with percent RSD in the range

Table 1: Limit of detection, limit of quantifications and retention times of the sixteen studied pesticides for rice grains

S.No.	Name of Pesticides	RTs (min)	In Rice		In Wheat	
			LOD*	LOQ*	LOD*	LOQ*
1	Trifluralin	8.02	5	20	N/A	N/A
2	α -BHC	8.63	1	2	1	2
3	β -BHC	9.42	1	3	1	3
4	Lindane	9.46	3	5	3	5
5	Delta-BHC	10.22	2	4	2	4
6	Malathion	12.57	10	50	10	50
7	Chlorpyrifos	13.02	20	50	20	50
8	Pendimethalin	14.39	20	50	N/A	N/A
9	Fipronil	14.65, 15.09	3	10	N/A	N/A
10	α -Endosulfan	16.21	4	50	4	50
11	Oxadiazon	17.74	50	100	N/A	N/A
12	Bifenthrin	20.73	10	50	10	50
13	Cyfluthrin	23.02	10	50	N/A	N/A
14	Cypermethrin	23.18, 23.29, 23.39	10	50	10	50
15	Fenvalerate	24.42, 24.71	10	50	14	50
16	Deltamethrin	25.51	14	50	5	5
17	λ -Cyhalothrin	21.08	N/A	N/A	20	5
18	Permethrin	22.18, 22.31	N/A	N/A	10	5

* = $\mu\text{g/kg}$

N/A = Not analyzed

Table 2: Percent recoveries of sixteen pesticides in rice and wheat grains at 100 and 500 $\mu\text{g/kg}$ fortification levels

S.No.	Name of Pesticides	Average percent recoveries and %RSD in Rice grain		Average percent recoveries and %RSD in Wheat grain	
		At 100 $\mu\text{g/kg}$ fortification level	At 500 $\mu\text{g/kg}$ fortification level	At 100 $\mu\text{g/kg}$ fortification level	At 500 $\mu\text{g/kg}$ fortification level
1	Trifluralin	85.33 \pm 12.42	89.00 \pm 8.48	N/A	N/A
2	Alpha-BHC	94.33 \pm 10.02	110.33 \pm 4.99	91.07 \pm 2.21	98.13 \pm 2.39
3	Beta-BHC	94.00 \pm 7.45	100.67 \pm 5.99	88.93 \pm 4.17	95.70 \pm 2.86
4	Lindane	90.33 \pm 6.76	103.67 \pm 6.28	88.83 \pm 2.98	91.40 \pm 2.29
5	Delta-BHC	97.67 \pm 5.25	104.33 \pm 7.32	91.57 \pm 3.06	95.13 \pm 4.60
6	Malathion	97.00 \pm 8.25	107.33 \pm 5.99	89.30 \pm 3.43	91.20 \pm 2.47
7	Chlorpyrifos	97.67 \pm 6.66	106.33 \pm 5.75	97.23 \pm 4.32	100.17 \pm 3.95
8	Pendimethalin	81.33 \pm 6.99	98.67 \pm 4.57	N/A	N/A
9	Fipronil	84.00 \pm 4.76	103.00 \pm 2.91	N/A	N/A
10	α -Endosulfan	74.00 \pm 9.74	109.00 \pm 8.15	83.20 \pm 4.64	89.40 \pm 2.79
11	Oxadiazon	98.00 \pm 9.35	100.00 \pm 8.89	N/A	N/A
12	Bifenthrin	93.67 \pm 8.29	107.00 \pm 7.65	87.17 \pm 9.27	93.63 \pm 3.58
13	Cyfluthrin	104.67 \pm 4.90	110.00 \pm 2.41	N/A	N/A
14	Cypermethrin	99.33 \pm 8.44	107.00 \pm 7.06	92.90 \pm 2.55	94.70 \pm 6.41
15	Fenvalerate	94.00 \pm 9.75	96.67 \pm 4.66	77.03 \pm 4.07	82.47 \pm 3.84
16	Deltamethrin	81.00 \pm 9.80	111.33 \pm 9.00	84.20 \pm 5.46	89.07 \pm 4.04
17	λ -Cyhalothrin	N/A	N/A	73.77 \pm 3.72	89.33 \pm 6.48
18	Permethrin	N/A	N/A	79.80 \pm 2.38	80.23 \pm 3.34

of 4.76 to 12.42 and from 89.00% to 111.33% recovery with % RSD in between 2.41 and 9.00 at 500 $\mu\text{g/kg}$ fortification level. However, in wheat grain samples, Recoveries were found in the range of 73.77% to 97.23% with %RSD in between 2.21 and 9.27 and 80.23% to 100.17% with %RSDs between 2.29 and 6.48 at 100 $\mu\text{g/kg}$ and 500 $\mu\text{g/kg}$ fortification levels respectively.

Gas chromatography with electron capture detector (GC-ECD) was found to be sensitive for all eighteen studied pesticides. Oven temperature was ramped for getting better separation of peaks of the studied pesticides. Total run time was 26 minutes. Under standardized GC parameters all the pesticides gave well resolved peaks both in wheat and rice as shown in Fig. 1.

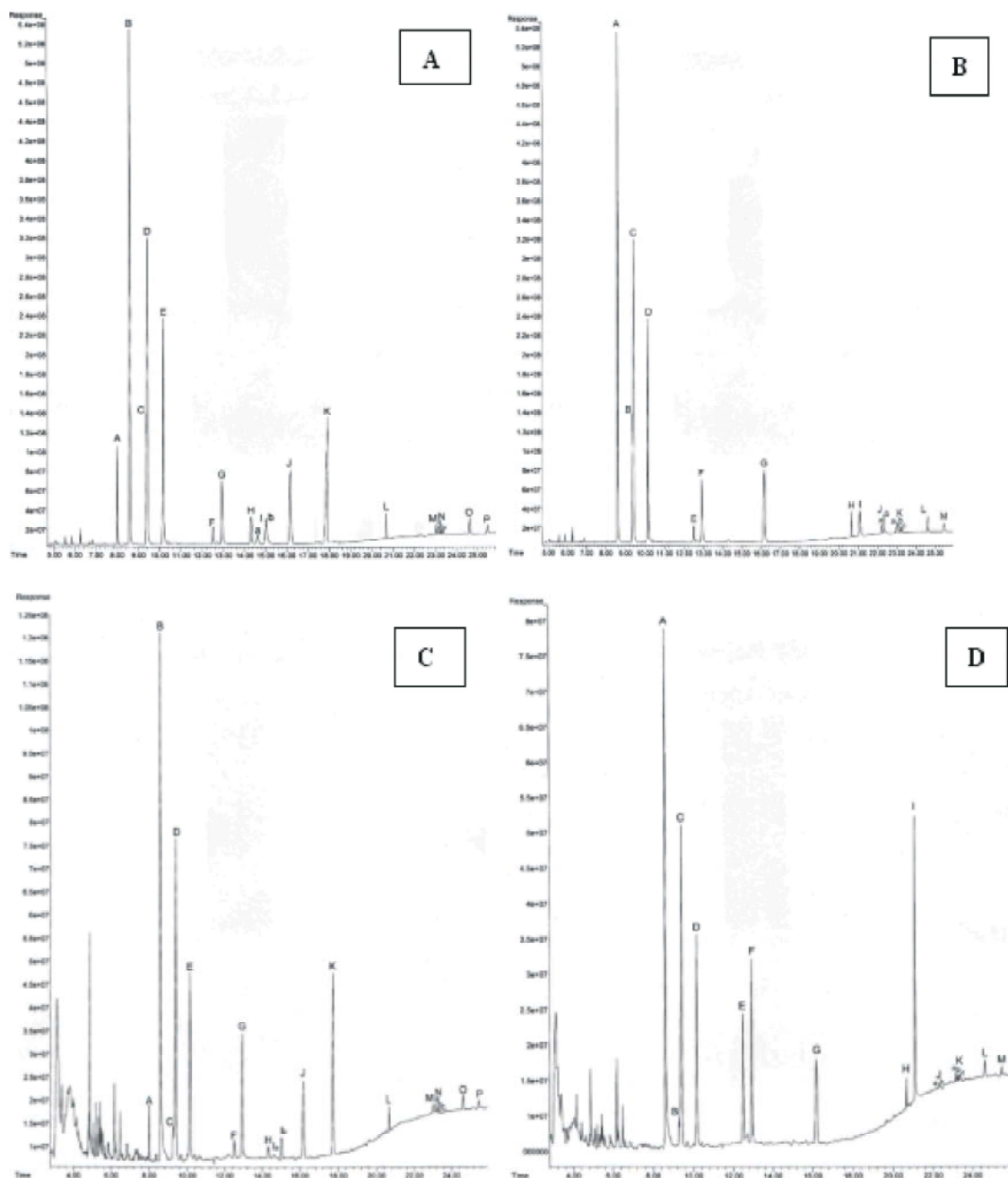


Fig. 1: GC-ECD Chromatogram: Separation of multiple pesticides in rice and wheat grain

A) Chromatographic separation of 16 pesticides including 3 herbicides standards (1 \square g each) for rice grain. B) Chromatographic separation of 13 pesticides standards (1 \square g each) for wheat grain. C) In Rice Grain: 16 pesticides including 3 herbicides (at 100 μ g/kg fortification level); A: Trifluralin, B: alpha-BHC, C: Beta-BHC, D: Lindane, E: Delta-BHC, F: Malathion, G: Chlorpyrifos, H: Pendimethalin, I, (a,b) Isomers of Fipronil, J: alpha-Endosulfan, K: Oxadiazon, L: Bifenthrin, M: Cyfluthrin, N: (a,b,c) Isomers of Cypermethrin, O: Fenvalerate, P: Deltamethrin. D) In Wheat Grain: 13 pesticides (at 100 μ g/kg fortification level); A: alpha-BHC, B: Beta-BHC, C: Lindane, D: Delta-BHC, E: Malathion, F: Chlorpyrifos, G: alpha-Endosulfan, H: Bifenthrin, I: Lambda-Cyhalothrin, J: (a,b) isomers of Permethrin, K: (a,b,c) isomers of Cypermethrin, L: Fenvalerate, M: Deltamethrin

Table 3: coefficients of determination of all studied pesticides

S. No.	Pesticides	R ² value	S. No.	Pesticides	R ² value
1	Trifluralin	0.9985	10	α -Endosulfan	0.9990
2	α -BHC	0.9993	11	Oxadiazon	0.9986
3	β -BHC	0.9992	12	Bifenthrin	0.9985
4	Lindane	0.9988	13	Cyfluthrin	0.9982
5	Delta-BHC	0.9997	14	Cypermethrin	0.9996
6	Malathion	0.9994	15	Fenvalerate	0.9993
7	Chlorpyrifos	0.9981	16	Deltamethrin	0.9991
8	Pendimethalin	0.9998	17	λ -Cyhalothrin	0.9986
9	Fipronil	0.9984	18	Permethrin	0.9980

Table 4: Residues of different pesticides found in wheat / rice samples

	Insecticide	N	Pesticide residues ($\mu\text{g/kg}$)	Origin	MRLs ($\mu\text{g/kg}$)
Wheat grain	Permethrin	2	31.0* (14.0 – 48.0)**	Imported	5000
	Malathion	2	71.1 (41.9 – 100.3)	Imported	8000
	Deltamethrin	4	639.2 (65.0 – 1290.3)	Domestic	2000
	Cypermethrin	2	170.4 (140.4 – 200.5)	Imported	1000
	Chlorpyrifos	3	128.6 (73.5 – 230.0)	Imported	1000
Rice grain	Deltamethrin	1	67	---	2000
	Fipronil	1	10	---	10
	Endosulfan	1	100	---	200

N = number of samples found contaminated

* Average - three replicates

** (minimum residues - maximum residues)

Table 5(a): FAPAS Proficiency Test number 0967, 2010 results in wheat flour

Laboratory No.	Carbendazim 179* $\mu\text{g/kg}$			λ -Cyhalothrin 102* $\mu\text{g/kg}$			Deltamethrin 188* $\mu\text{g/kg}$		
	Result $\mu\text{g/g}$	% recovery	Z-score	Result $\mu\text{g/g}$	% recovery	Z-Score	Result $\mu\text{g/g}$	% recovery	Z-score
26	≠			140.0	87.4	1.6	210.0	79.4	0.6

≠ = Pesticide not analyzed

* = Assigned concentration

Table 5(b): FAPAS Proficiency Test 0969, 2011 results in rice test material

Laboratory No.	Azoxystrobin 234* $\mu\text{g/kg}$			Carbaryl 248* $\mu\text{g/kg}$			Malathion 258* $\mu\text{g/kg}$			Pirimphos-methyl 225* $\mu\text{g/kg}$		
	Result $\mu\text{g/g}$	% recovery	Z-score	Result $\mu\text{g/g}$	% recovery	Z-Score	Result $\mu\text{g/g}$	% recovery	Z-score	Result $\mu\text{g/g}$	% recovery	Z-score
31	≠			≠			299.83	119.85	0.8	≠		

≠ = Pesticide not analyzed

* = Assigned concentration

However, beta BHC was shown overlapped somewhat on Lindane (gamma BHC). Linearity of the gas chromatographic system was checked and determined by plotting different concentrations (i.e. 0.01 to 10.0 $\mu\text{g/g}$) of all pesticides against their respective response-area (Table 3). Calibration curves were found linear with coefficients of determination (R^2) > 0.998.

In pesticide residues analysis, adsorption chromatography has been among the most frequently employed cleanup approaches for many decades, with

numerous existing methods, this is still using throughout the world. Therefore, in the present study adsorption chromatography was applied in which a mixture containing activated graphitized charcoal and aluminum oxide (acidic) exhibits the best cleanup. In case of improper cleanup the high molecular weight co-extractive compounds of samples may be deposited at the tip/edge of the injection port and/or capillary column of the GC that results in reduced-efficiency of chromatographic separation and in turn induce unreliable results [15].

Using electron capture detector (ECD) system, cleanup is necessary and recommended. Gas chromatographic system especially ECD may be rapidly deteriorated due to insufficient cleanup and ultimately prohibit the reliable results [2]. In this study, the acidic aluminum oxide was found the best in its capacity to retained oily materials and other high molecular weight matrix organic compounds of the samples while activated graphitized charcoal retained coloring co-extractives. No interfering peak in the control samples of both rice and wheat was observed in the study region.

Worldwide recognized standard criteria for the reliability (acceptability) of analytical method for the detection of multi-residue is the ability of method to produce mean average recovery between 70% and 110% with $RSD \leq 10\%$ [16,17]. From fortified samples, mean average recoveries results obtained in this study were also found within the acceptable range.

The developed method was used for the monitoring of multi-residue pesticides in two hundred samples of rice and wheat received from exporters & importers included local (domestic) stored wheat grains collected from public storage main godowns situated in Pepri and Landhi areas of Karachi, Pakistan. Detected pesticides residues results are indicated in Table 4.

In rice samples, three insecticides (Deltamethrin, Endosulfan and Fipronil) residues were detected in individual sample each. However, all the residual quantities were found to be much lower than maximum residual limits (MRLs). In the wheat grains samples, pesticides residues were found in 22.5% of the imported samples while in domestic stored wheat grains, the residues of deltamethrin were detected in 6.7% samples. All the residues found were below the MRLs of FAO/WHO [18].

The method was also validated by participating for Proficiency Tests (PTs) in 2010 (for wheat grains) and in 2011 (for rice grains) to confirm the reliability of the results. The tests were offered by Food Analysis Performance Assessment Scheme (FAPAS). Under this scheme, the closeness of the reported results with assigned (true) value was represented with reference to Z-score [19] that is considered to be a significant and the most reliable assessment score for laboratories methods validation proposed. The obtained results of wheat and rice grains have been depicted in Table 5a and 5b, respectively along with the assigned laboratories number by FAPAS.

As compared to the previously reported method [2], the present developed procedure has four advantages:

After the eluent was passed through chromatographic glass column (charcoal/acidic alumina), mini-column cleanup step is not required. Rather using two separate GC operating parameters for synthetic pyrethroid and OPs (organophosphorus) pesticides in the same sample, the present method has detection efficiency of synthetic pyrethroid, chlorinated and organophosphorus pesticides in a single injection. Khan *et al.* [2] method was restricted for the analysis of nine insecticides of synthetic pyrethroids and OPs, whereas the present method has the efficiency of analyzing sixteen multi-group pesticide residues including both insecticides and herbicides. The current method has also been validated through proficiency tests, while these tests have never been practiced for the method of Khan *et al.* [2].

CONCLUSIONS

Recoveries of the studied insecticides and herbicides were determined by spiking at low concentration level of 100µg/kg and at high concentration level of 500µg/kg in both wheat and rice grains. Average lowest mean recoveries in wheat were found in the range between 73.77% to 80.23% with %RSD values 3.72 and 3.34 at 100 and 500µg/kg, respectively. In rice, the average lowest mean recoveries found at low and high concentrations, were 74% to 89% with %RSD 9.74 and 8.48, respectively. Good linearity of the calibration curves were obtained with a co-efficient of determination > 0.998 . Method development carried out only through determination of average recovery is indeed incomplete and could in principle be misleading. Therefore, in this study, the developed method was validated both for rice and wheat by participating in the FAPAS® proficiency tests and successfully qualified by getting the desired Z-score values. Keeping in view the outcome of these results, the reported method has been tested and found suitable, reliable and efficient for the analysis of multi-group residues of pesticides in wheat and rice. However, there are several highly sophisticated methods and instruments for extraction and analysis of pesticide residues in the developed countries. But, they demand high running cost and the developing countries face difficulties in complete adaptation for such routine monitoring studies.

Therefore, this reported procedure can easily be adopted in such laboratories those have no opportunities and access to modern extraction techniques and sophisticated instruments like GC-MS.

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