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Research Article

OCCURRENCE OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN POULTRY FEED AND MEAT

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ABSTRACT

In the present study, gas chromatography with nitrogen phosphorus detector (GC-NPD) technique was standardized for the simultaneous detection and quantitation of organophosphorus pesticide (OP) residues i.e. chlorpyrifos, malathion and profenofos from poultry feed and meat. The standardization procedure showed that the values for the system precision (% RSD for all analytes was <0.07% for area and 0.02% for retention time), linearity (r^2>0.99), specificity and accuracy (88.5-92.1% and 85.2-90.5% in feed and meat, respectively) were within accepted range and demonstrated system suitability for analysis of samples. The determination of OP pesticides was carried out in the 50 randomly collected samples each of poultry feed and meat (n=100) collected from poultry farms in and around Hisar district (Haryana). Mean concentrations of chlorpyrifos pesticides in poultry feed and meat were 20.614 and 6.587 µg/kg, respectively, while that of malathion were 6.846 and 1.994 µg/kg, respectively. Mean concentration of profenofos in poultry feed was 572.783 µg/kg but it was not detected in any of the poultry meat samples. Comparison of pesticide concentration in each positive sample of meat with national and international MRLs showed that, chlorpyrifos was responsible for violations in two samples. It was concluded that the poultry feed may be a significant source of pesticide residues in poultry meat.

Key words: pesticide residues, chlorpyrifos, malathion, profenofos, poultry, feed, meat

Wide spread use of pesticides in the production system in agricultural and allied sector, persistence in the environment and their varying toxicity make them a major component of public health consideration. Now a days, more than 800 different kinds of pesticides formulations are used for the control of insects, fungi and unwanted plants in the process of agricultural production. Although most of them leave the products or degrade in soil, water and atmosphere, some trace amounts of pesticide residues can be transferred to human via the food chain, being potentially harmful to human health (Benbrook, 2002). Perusal of the residue data on pesticides in samples of fruits, vegetables, cereals, pulses, grains, wheat flour, oils, eggs, meat, fish, poultry, bovine milk, butter and cheese in India indicates their presence in sizable amounts (Bhushan, 2006). Foods of animal origin have maximum contamination by pesticides followed by leafy vegetables and garden fruits (Rathore *et al*., 1996).

Organophosphorus pesticides (OP) have been used extensively as insecticides, fungicides, herbicides and animal pesticides for more than four decades. The use of these chemicals in place of organochlorine pesticides is attributed to their less persistent characteristics in the environment. Though OPs are biodegradable, presence of a few of them has been validated in oil seeds (Singh *et al*., 1998), rice and rice bran (Chinniah *et al*., 1998). In intensive poultry production, the poultry feed can act as important source of pesticide residues (Khilare *et al*., 2016). In India especially Haryana, there is a paucity of reports related to occurrence of pesticide residues in poultry feed/ feed ingredients and meat. Therefore, the present investigation was planned with the objective to standardize the gas chromatography (GC) technique for detection and quantification of OP residues (chlorpyrifos, malathion and profenofos) in poultry feed and meat.

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MATERIALS AND METHODS

Collection of Samples: The present work was carried out in the Department of Veterinary Public Health and Epidemiology, LUVAS, Hisar. For this, 50 samples each of poultry feed/feed ingredients and chicken meat were randomly collected in transparent polyethylene bags from various poultry farms in and around Hisar district of Haryana (total 100 samples). Samples were stored at -20°C till analysis.

Chemicals and Reagents: Pesticide reference standards *viz.* chlorpyrifos, malathion and profenofos (purity >98.0%) and HPLC grade solvents and chemicals *viz.* Celite 545 and calcium silicate, acetone, acetonitrile, ethyl acetate and n-hexane was procured from, Sigma Aldrich, U.S.A. Anhydrous sodium sulphate was procured from M/s Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Preparation of Reagents: Reagents were prepared and calibrated as per the instructions given in Pesticides Residues Manual (AMPRF, 1996). Stock solution of each pesticide (primary standard solution) was prepared in n-hexane. For preparation of working standard solutions, the maximum residue limits (MRLs) prescribed by Food Safety and Standard Authority of India (FSSAI, 2011), European Union (EU, 2006), Codex Alimentarius Commission of WHO (Codex, 2006) were considered depending upon their existence. Based on the MRLs, a linearity range (50, 100, 150, 200 and 250 ng/ml) was selected to cover the lowest MRL prescribed. Then appropriate dilutions of secondary standard solution in n-hexane were made to produce a required dilution of working solution.

Sample Extraction and Cleanup: Laboratory method for detection of pesticide residues in feed and meat was standardized as per Pesticide Residue Manual (AMPRF, 1996). Briefly, sample was triturated with Sodium sulfate and extracted with acetonitrile and acetone in the presence of adsorbants, celite and calcium silicate. The extract obtained was evaporated to dryness and re-dissolved in n- hexane and cleaned up by liquid-liquid extraction with acetonitrile. Acetonitrile portion was collected and reconstituted in n-hexane after complete evaporation before subjecting it to gas chromatographic analysis.

Chromatographic Analysis: A Shimadzu gas chromatograph model GC 2010 Plus equipped with a programmed temperature vaporizer (PTV) injection port (manual injection) connected to nitrogen-phosphorous detector through Equity 1 capillary column (30 m x 0.25 mm I.D.) was used for analysis of OP pesticides. Initial temperature of column was adjusted to 60°C and held for 2 min., and then temp was raised at the rate of 20°C/ min to 210°C held for 2 min, then 5°C/min to 250°C held for 5 min. The total run time was 24.5 min. Split ratio was kept at 1:14 with column flow at the rate of 1.47ml/ min. Injector and detector temperature was set to 250°C and 300°C respectively, and the injection volume was1µl.

Standardization and Validation Studies of Gas Chromatography Technique: The system precision was evaluated by studying the reproducibility of the instrumental response with respect to retention time and area of an analyte. Percent Relative Standard Deviation (%RSD) for all analytes was found to be less than 0.07% for area and 0.02% for retention time. Specificity was evaluated by visual observation of chromatograms of blank sample matrix and sample matrix spiked with standard mixture. For poultry feed and meat, chromatographic signals at the retention times of pesticides were absent in blank sample matrix.

The standard calibration curves of the analyzed OP pesticides presented a good regression line (r^2>0.99) in the range of explored concentrations i.e. from 50 to 250 ηg/ml. The graphs showing calibration curve of these pesticides, revealed that all concentrations of the OP pesticides under study were collinear and thus calibration curves were further employed for the detection of analytes under study. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined on the basis of standard deviation of the blank. Measurement of the magnitude of the analytical background response was performed by the analysis of 10 blank samples and calculating the standard deviations of these responses. LOD was calculated by formula: $\overline{X} + 3\sigma$ and LOQ by formula: $\overline{X} + 10\sigma$.

Table 1 summarizes the LOD and LOQ obtained for each pesticide and for poultry feed and meat in OP pesticide group. Perusal of table clearly indicated that the LOD and LOQ for individual analytes were well below their respective MRLs indicating that the method was able to detect the given pesticide at sufficiently low level.

The accuracy in terms of percent recovery of each pesticide in OP pesticide group at five different fortification levels (50, 100, 150, 200 and 250 µg/kg) was evaluated for poultry feed and meat and the results are presented in Table 1. Satisfactory results were found in almost all instances. Recoveries for all analyte-matrix combinations ranged between 88.5-92.1% in feed and 85.2-90.5% in

RESULTS AND DISCUSSION

Table 1
Method performance parameters for detection of residues in poultry feed and meat for OP pesticides

Analyte	Limit of Detection (mg/kg)		Limit of Quantitation (mg/kg)		Accuracy (Average recovery %)		Precision (Average CV %)	
	Feed	Meat	Feed	Meat	Feed	Meat	Feed	Meat
Chlorpyrifos	0.0037	0.0027	0.0100	0.0069	92.133	85.207	5.224	8.923
Malathion	0.0025	0.0027	0.0069	0.0068	88.925	88.703	10.519	9.790
Profenofos	0.0059	0.0024	0.0157	0.0059	88.517	90.549	6.752	7.189

Table 2
Number of poultry feed and meat samples positive for OP pesticide residues

Pesticides	Feed (n=50)	Meat (n=50)
Chlorpyrifos	3 (6%)	2 (4%)
Malathion	2 (4%)	1(2%)
Profenofos	4 (8%)	ND

meat. However, in general, the pesticides gave acceptable recoveries within the mentioned validation interval as per EU legislation (EU, 2002) i.e. between 70 and 110 percent. The precision of the method was assessed at five concentration levels (50, 100, 150, 200 and 250 µg/kg) on the basis of recovery studies on spiked samples. Repeatability and intermediate precision values, expressed as relative standard deviation (CV percent) were found less than 10.51 for OP pesticides (Table 1).

Overall, the multiresidue method followed for simultaneous detection and quantification of OP pesticide residues in poultry feed and meat was subjected to rigorous validation parameters. The system precision values indicated a good consistency in response by the GC instrument used during present study. A good linearity was noted for standards and spiked poultry feed and meat samples. The absence of interfering peaks in blank sample indicated good specificity for extraction and clean-up of the method used in present study. Accuracy and precision of the method were in accepted range in comparison with international guidelines (EU, 2002). These results of validation studies clearly demonstrated that the present method is suited for routine analysis of OP pesticides from poultry feed and meat samples.

OP Pesticides Residues in Poultry Feed and Meat: Validated method was applied for detection of OP pesticides residues in 50 samples each of poultry feed and meat collected from poultry farms in and around of Hisar. The overall occurrence of OP pesticide residues is presented in Table 2.

Mean concentration along with range for each pesticide in poultry feed and meat is provided in Table 3. The results revealed that mean concentrations of chlorpyrifos in poultry feed and meat were 20.614 and 6.587 µg/kg, respectively. While that of malathion were 6.846 and 1.994 µg/kg, respectively. Mean concentrations of profenofos in poultry feed were 572.783 µg/kg but it was not detected in any of the poultry meat samples. In the present study, overall occurrence of chlorpyrifos was found to be 6 percent in poultry feed and 4 percent in poultry meat.

Reports of detection of chlorpyrifos residues in poultry feed and meat are scanty. Nagra (2006) found residues of chlorpyrifos with mean levels of 0.09 mg/kg in poultry feed from Punjab, India. Poultry feed are typically composed of ingredients like wheat, rice, and maize as source of energy. Skrbic (2007) found 422- 1336 ng/g in wheat samples collected from Serbia and Chen *et al.* (2009) detected 0.011–1.756 mg/kg in rice samples collected from China. Further, toxicity studies of chlorpyrifos have shown that, at its residual concentration in food, the harmful effects on human and animals are unlikely (EXTOXNET, 1996). Therefore, findings of the present study revealed that although chlorpyrifos residues showed occurrence in poultry meat, its presence at quite low concentration may not be a major food safety issue for poultry meat consumers. Occurrence of malathion was found to be 4% in poultry feed and 2% in poultry meat. The mean concentration of malathion was 0.076 ìg/kg in poultry meat and 0.252 ìg/kg in poultry feed. The available literature indicates paucity of reports on presence of malathion residues in poultry meat. In India, Jadhav (2008) reported 6.66 ìg/kg malathion in poultry meat samples collected from Maharashtra. The findings of present study with respect to malathion showing quite low occurrence with low mean concentration in poultry meat matches with its fate in environment and animal's body. Its presence in feed may be due to approved use of malathion for control of pests during storage of maize and other grains which are common ingredients.

Overall presence of profenofos in poultry feed was found to be 8 percent which was highest as compared to other OP pesticides studied, where as in poultry meat, profenofos pesticide residues were found below detectable

Table 3
Mean concentration of OP pesticide residues in poultry feed and meat

Pesticides	Mean conc. on wet wt. basis (µg/kg)		Mean conc. on fat wt. basis (µg/kg)		Range conc. on wet wt. basis (µg/kg)		Range conc. on fat wt. basis (µg/kg)	
	Feed	Meat	Feed	Meat	Feed	Meat	Feed	Meat
Chlorpyrifos	1.298	0.181	20.614	6.587	9.625-6.017	2.989-6.017	98.583-638.235	96.575-232.766
Malathion	0.252	0.076	6.846	1.994	4.049-8.551	3.834	81.224-261.099	99.714
Profenofos	20.012	ND	572.783	ND	16.833-612.421	ND	135.531-20655.01	ND

ND=Not detected

Table 4
Comparison of OP pesticide residue levels in poultry meat field samples with national and international MRLs

Analyte	Number of samples with violative MRL concentration (mg/kg)		
	FSSAI	Codex	EU
Chlorpyrifos	1	2	2
Malathion	NA	NA	1
Profenofos	NA	ND	ND

NA=Not applicable; ND=Not detected

limit. The information on detection of this pesticide in poultry feed and meat is lacking. The absence of residues in poultry meat samples indicate that profenofos may not be of important concern for this commodity.

Comparison of OP Pesticide Residues with MRLs: The results showing number of samples with violative MRL concentration are presented in Table 4. With respect to OP pesticides, the FSSAI and Codex MRLs are not available for malathion. As well as, the FSSAI MRLs are not available for profenofos. Violative concentration of chlropyrifos was found in 2% (as per FSSAI) and 4% (as per Codex and EU MRLs) meat samples. As per the EU MRLs for malathion, 2% samples showed violative concentration.

Overall, based on frequency of detection and concentration of various analytes of OP pesticides studied in the present work, it can be stated that, with exception of profenofos, poultry meat samples were laden with all other pesticides included in the study. The rate of accumulation in poultry meat was correlated well with that of rate of occurrence in poultry feed. From the findings of the present study it can be concluded that, the poultry feed may be a significant source of chlorpyrifos and malathion pesticide residues in poultry meat. Although as per FSSAI MRLs, there appeared no hazard by consuming poultry meat for Indian consumers. However, further monitoring studies are required to produce residue free meat for domestic consumers and exports.

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