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UHPLC determination of residues of chlorpyrifos insecticide in chicken meat

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Abstract

The present study was therefore aimed to determine the residual concentration of one of the most commonly used insecticides chlorpyrifos in meat samples of chicken, on the basis of surveillance at various poultry farms in and around Jabalpur district. A total number of 150 meat samples were collected from different poultry farms and local market in and around Jabalpur. The extraction procedure was based on liquid-liquid partition and clean-up procedure by alumina column technique. Acetonitrile and water were used as mobile phase in the ratio of 80:20 (v/v) for UHPLC determination. The limit of detection and limit of quantification for chlorpyrifos was as 0.014 mg/kg and 0.0463 mg/kg, respectively. The recoveries of chlorpyrifos in meat samples found in the range of 82-94 per cent and none of the analyzed samples were found positive for residual concentration of chlorpyrifos in meat samples.

Keywords: Insecticide, chlorpyrifos, chicken, acetonitrile, UHPLC

1. Introduction

Pesticides use in agriculture is the most economical approach to control various pests, though they are considered major contaminant of our environment [16]. Livestock generally accumulate persistent organic pollutants from contaminated feed and water or from pesticide application in animal production areas [12]. Poultry can also be contaminated by feeding on plant materials that have been treated with pesticides during the growing and/or storage stages. Consequently, chickens and hens accumulate residues in muscles, fat, and liver as well as residues can also be detected in eggs, even long after the chemicals have been eliminated from the other tissues of the laying hens [4, 5, 14]. The chronic effects of pesticides from food intake on human health are not well defined, but there is increasing evidence of carcinogenicity and genotoxicity, as well as disruption of hormonal functions [1, 3]. Since diet is the main source of chronic exposure to low doses of these substances, humans are mainly exposed through ingestion of meat or other meat products [7, 10, 15]. Chlorpyrifos, an organophosphorus (OP) compound is widely used to improve the yield of agricultural produce in India. Public concern over pesticide residues in vegetables, fruits, and food of animal origin has been increasing and became a significant food safety issue. The poultry industry in Jabalpur has an extensive network of broiler production poultry farms throughout the district. The present study is therefore aimed to determine the residual concentration of chlorpyrifos in meat samples of chicken.

2. Materials and Methods

The collection of data about use of various pesticide/insecticide was based on surveillance study at 50 poultry farms and agricultural fields located in and around Jabalpur, therefore, one of the most commonly used insecticides chlorpyrifos was shortlisted for study. On the basis of data, 10 organized broiler poultry farms from five target areas (JBP-1 to JBP-5) were selected and denoted as T₁ to T₁₀. A total of 150 meat samples including muscle, liver and kidney were collected from different target poultry farms as well as their local market area. Samples were processed and analyzed for the determination of concentration of chlorpyrifos residues on the day of collection by using Ultra High Performance Liquid Chromatography (UHPLC) apparatus made of Shimadzu Corporation, Japan. UHPLC assembly is equipped with binary gradient solvent delivery pump (SIL-30AC) with PDA detector (SPD-M20A.). Column A Li Chro CART® 250-4/Li Chrospher® 100 RP-18e endcapped (250×4 mm, with particle size of 5 μm) column was used. Chromatographic separation was performed using C₁₈ reverse phase column (Supelco Discovery column 25cm x 4.6 mm, particle size 5μ). Chlorpyrifos standard was purchased from Sigma-Aldrich with 99.6% purity produced from Sigma-Aldrich GmbH, Industries, Switzerland. Solvents like acetonitrile, water and dichloromethane were HPLC

grade, from Chromasolv® (Sigma - Aldrich) and anhydrous sodium sulphate and magnesium sulfate from Merck (AR grade) were used.

Extraction and clean-up procedure of meat samples based on liquid-liquid partition and alumina column chromatography, followed the methodology done by Bottomley and Baker as per standard protocol with some suitable modifications [12].

a) Preparation of meat samples

Five g of meat sample (muscle, liver and kidney) was homogenized with addition of 20 ml acetonitrile in a 25 ml test tube and kept it on mechanical shaker for half an hour to shake well. Centrifuged the homogenized mixture at 7,000 rpm at 4°C for 10 minutes. Filtered the supernatant and transferred in a separatory funnel for Liquid-liquid partition with sodium sulfate solution, 40ml (2.5%) and dichloromethane (10ml). Lower layer was collected in a test tube; repeated partition two times then added anhydrous sodium sulfate (4g) and evaporated till drying by using lyophilizer. The dry residue was cleaned by using alumina column eluted with 20 ml dichloromethane. The cleaned samples were again evaporated completely till dryness. The dried residue was reconstituted with 1 ml acetonitrile and filtered through 0.22 µm nylon syringe filter. The 20µl of filtrate was put in auto-sampler of UHPLC for analysis.

b) Method validation

Standard operating procedure for liquid chromatographic conditions was employed for both insecticides. Acetonitrile

and water were used as mobile phase in the ratio of 80:20 (v/v) and flow rate for the mobile phase was 1.0 ml/min. and the temperature of column oven was 40°C. Detection was performed with UV diode array detector (UV-DAD) and retention time was recorded. Calibration curve was obtained by serial dilution of standards in acetonitrile at different concentrations with 2.0, 1.0, 0.1, 0.05, 0.02 and 0.01 ppm to get the linear range (Linearity). The mean correlation coefficient (r^2) was recorded and each point of calibration curve represented mean of three replicates. Recovery analysis of chlorpyrifos was carried out in meat samples, spiked at 10, 5 and 1.0 ppm of insecticide standards. The recovery values were calculated as the ratio between the observed and spiked concentrations. Sensitivity of UHPLC method was determined from limit of detection (LOD) and limit of quantitation (LOQ), were calculated as the concentration corresponding to a signal to noise (S/N) ratio of 3. The limits of quantitation (LOQs) were calculated as the concentration corresponding to a signal to noise (S/N) ratio of 10.

3. Results and Discussion

The analysis of chlorpyrifos in chicken meat (muscle, liver and kidney) was done by UHPLC method. The mobile phase solvent system exhibited sharp peaks as chromatogram and purity line at different concentration levels for analyte. The mobile phase exhibited good separation of chlorpyrifos from its matrix with a mean retention time of 6.33 minutes (Fig. 1), the maximum absorbance was recorded at 229 nm.

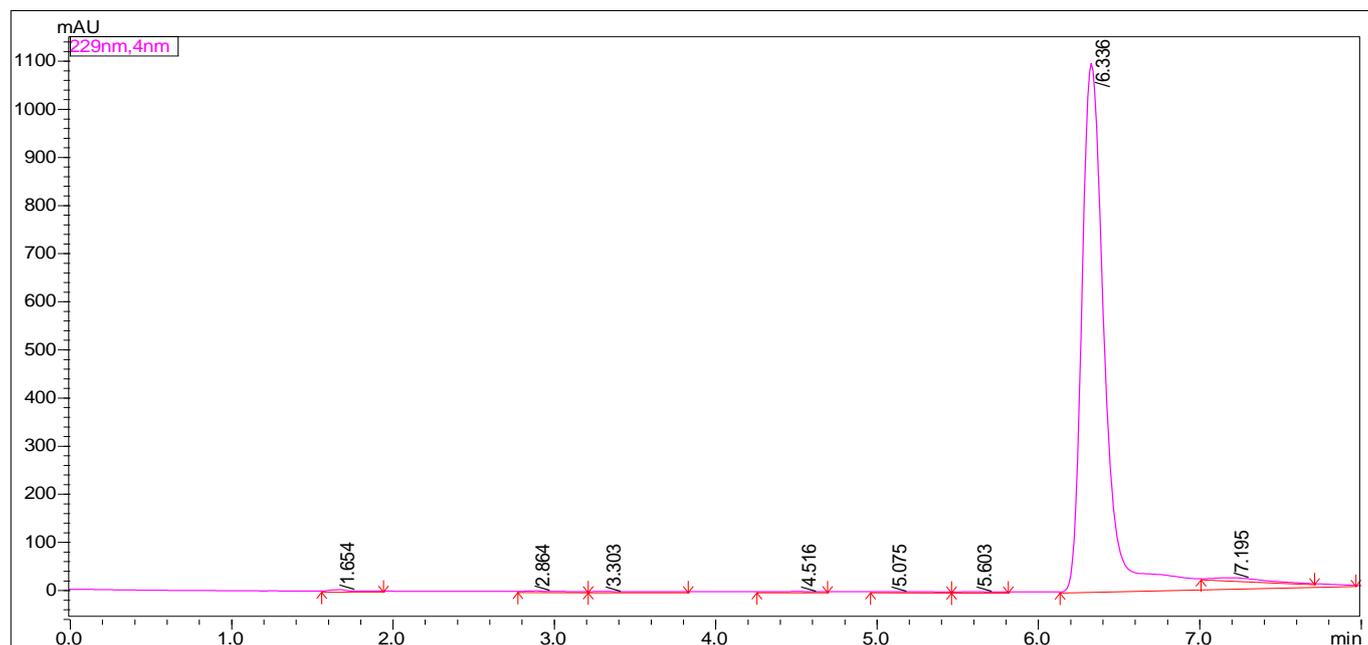


Fig 1: Representative chromatogram of chlorpyrifos at 1ppm with 6.33 minutes retention time.

A total numbers of 150 chicken meat samples were analyzed including 15 samples from each 10 target farms from Jabalpur-1, Jabalpur-2, Jabalpur-3, Jabalpur-4 and Jabalpur-5 target areas. There is no any residual concentration could be detected in all meat samples analyzed for chlorpyrifos insecticide (Fig. 2) and (Table 1). However, Muhammad and co-workers analyzed chlorpyrifos in meat samples collected from different regions and revealed that 21 per cent of meat samples were contaminated with chlorpyrifos [9]. The absence

of residual concentration of insecticide indicated their short half-life and rapid metabolism from body of chicken and degradation from stored plant feed materials. The aforesaid reports of various researchers clearly indicated that chlorpyrifos has relatively lesser half-lives and therefore the analytes could not be detected in poultry meat if proper withdrawal period is maintained as well as if plant material is stored for long time.

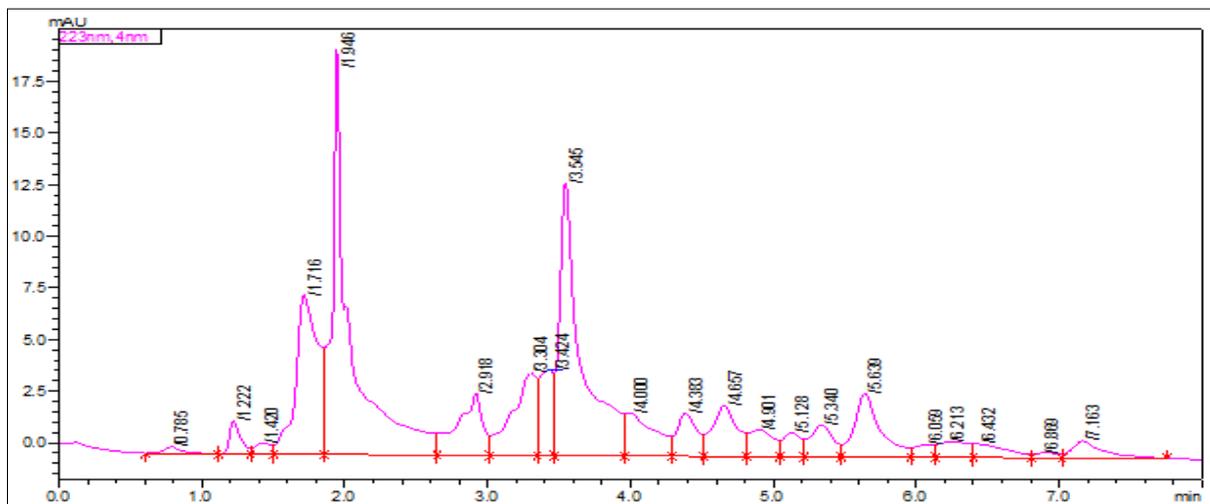


Fig 2: Representative chromatograph showing undetectable concentration of chlorpyrifos in meat samples at 6.33 minutes of retention time.

Table 1: Mean residue concentration of chlorpyrifos (mg/kg) in chicken muscle, liver and kidney samples of different target areas

Target area	No. of samples	Mean residual concentration(mg/kg)			
		Muscle	Liver	Kidney	
Jabalpur-1	T1	15	ND	ND	ND
	T2	15	ND	ND	ND
Jabalpur-2	T1	15	ND	ND	ND
	T2	15	ND	ND	ND
Jabalpur-3	T1	15	ND	ND	ND
	T2	15	ND	ND	ND
Jabalpur-4	T1	15	ND	ND	ND
	T2	15	ND	ND	ND
Jabalpur-5	T1	15	ND	ND	ND
	T2	15	ND	ND	ND

ND: Not Detected

The recovery of chlorpyrifos in chicken meat samples were in the range of 82-94 per cent. The recoveries of chlorpyrifos in

meat samples showing maximum to the extent of 94 per cent in meat samples is in accordance with the recovery percentage as exhibited by Nagappa and Singh [11] who also determined chlorpyrifos recovery to 90.7 per cent in meat samples by using HPLC and present study is also close agreements to the study of Kumar and co-workers who reported the recovery to the extent of 86.17 per cent by using GPLC [8]. Recovery of chlorpyrifos. Experiment of the present study demonstrated high accuracy and precision of the technique used in terms of linearity showing correlation coefficient (r^2) of chlorpyrifos as 0.9998 (Fig. 3). This clearly indicates that SOP of UHPLC was precise and accurate. The linearity of chlorpyrifos was recorded by Zalat and co-workers by using HPLC who plotted the graph and calculated the linearity (r^2) as 0.9998, which substantiates our findings on linearity criteria [17]. A study by Jagadish and co-workers found the linearity of chlorpyrifos to the extent of 0.99 for analyte which also supports our findings indicating the linearity was excellent [6].

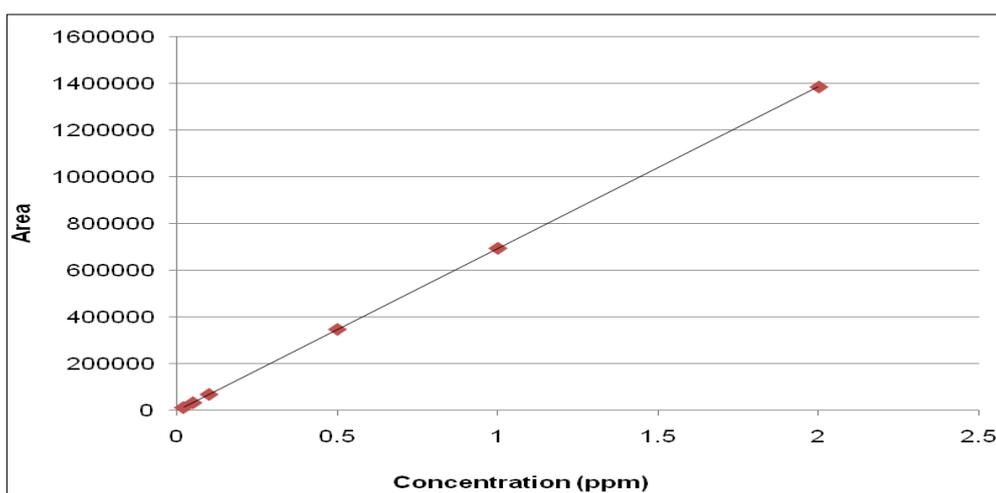


Fig 3: Linearity (Calibration) curve of chlorpyrifos

The sensitivity of UHPLC technique is determined on the basis of limit of detection (LOD) and limit of quantitation (LOQ) equations. The technique used in the present study showed good response in term of LOD calculated to be 0.014 mg/kg for chlorpyrifos. The developed method clearly suggests an appropriate limit of detection of chlorpyrifos by using UHPLC. The limit of detection for chlorpyrifos was also reported by Jagdish *et al.* (2015) as 0.02 $\mu\text{g/g}$ for

chlorpyrifos which is in close agreement to the finding of the present study. Sasikala and co-workers reported their findings of chlorpyrifos showing LOD of 0.02 $\mu\text{g/g}$ is in also close agreement to the finding of the present study [13].

4. Conclusion

Exposure of pesticides/insecticide occurs mainly through eating food of plant and foods of animal origin. Present study

was undertaken to determine residual concentrations of chlorpyrifos in meat (n=150) samples of chicken, collected from various poultry farms of Jabalpur district. No residual concentration was detected in meat samples, recommended by FAO/WHO guidelines. The present standard operating procedure showed good precision, high sensitivity and accuracy for detection of organophosphate insecticide in chicken meat.

5. Acknowledgement

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