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Evaluation of Oxytetracycline residues in Chicken Meat Samples by HPLC

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Abstract
Antibiotics are often used for therapeutic, prophylactic, and growth-promoting purposes. Antibiotics and their metabolites can accumulate in different tissues such as muscle, liver, and kidney if they are used indiscriminately. Consumption of treated bird meat during the withdrawal phase will expose customers to health risks such as antibiotic resistance. The present study was carried out to develop a simple and sensitive method for the determination of oxytetracycline (OTC) residue in poultry meat. The present study was aimed to assess the residue level of these antibiotics in chicken meat. Chicken meat samples (including muscle, liver, kidney and fat) from poultry farms and retail market were collected. High Performance Liquid Chromatography (HPLC) was used for screening of OTC. The analysis revealed that 18.89 % meat samples were positive for OTC. Out of it, 33.33 % samples were having concentration above the MRL for OTC. So it can be concluded that the usage of OTC in chicken lead contamination of meat if withdrawal period of drug not following and it may cause resistance in consumers and seems to be a public health threat.

Keywords Oxytetracycline (OTC), Antibiotic residue, HPLC, Uttarakhand

Introduction
Antibiotics are medications that are used to cure and avoid infections caused by bacteria (Black, 1977) [1]. They work by interrupting essential bacterial processes, destroying or slowing the bacteria's growth. The tetracycline antibiotic class is generally used in veterinary medicine to treat a number of infections, among which oxytetracycline (OTC) is the most commonly used. Antibiotics/metabolites accumulate in different tissues such as muscle, liver, and kidney due to uncontrolled use of these medications not only for therapy but also for prophylaxis (Alhendi *et al.*, 2000 and Abdel-Mohsein *et al.*, 2015) [2, 3]. Consumption of meat from treated animals during the withdrawal period presents a range of health threats to users, including antibiotic resistance, allergic reactions, and toxicity (Czeizel and Rockenbauer, 2000; Salama *et al.*, 2011) [4, 5]. The acceptable Maximum Residue Limit (MRL) for OTC as recommended by the joint FAO/WHO Expert Committee on Food Additives (2002) is 0.2 mg/kg in muscle (cattle, pig, sheep and poultry), 0.6 mg/kg in liver (cattle, pig, sheep and poultry), 1.2 mg/kg in kidney (cattle, pig, sheep and poultry) and 0.4 mg/kg in poultry egg (Walker and Ayres, 1958) [6]. The poultry industry is a rapidly growing animal husbandry sector. Antibiotics are widely used as medicinal, prophylactic, and growth stimulating agents in commercial farming, and they can remain in various tissues of birds if slaughtered until the withdrawal time ends. The present study was aimed to assess the residue levels of the antibiotic in chicken meat and compare with the permissible Maximum Residue Limits (MRL) in different districts of Uttarakhand. By using a powerful separation technique, such as HPLC, coupled with a UV detector and reverse phase column.

Materials and Methods
Sample collection
A total 254 chicken (35-45 days old) meat samples (including muscle, liver, kidney and fat) were collected from poultry farms and retail market in different districts of Uttarakhand over a period of one year.

Chemicals and reagent
Standard oxytetracycline dihydrate was obtained from Himedia. HPLC grade methanol, acetonitrile and oxalic acid were obtained from Merck. Oxytetracycline. Analytical grade Na_2HPO_4, EDTA and citric acid obtained from Merck, Germany. High purity Milli-Q water

generated in the laboratory was used for the study.

Standard preperation

Standard solution was prepared by dissolving 1mg of OTC standard powder in 1ml of mobile phase. Stock standard solutions were filtered and and stored at 4ºC. Working standard was prepared by serial dilution.

Sample treatment

The samples were kept at -20 degree centigrade until analysis. Analyzing of samples was carried out using 5g of either kidney liver muscle or fat. In each case sample were allowed to defrost at room temperature. Then tissues were homogenized and probe was rinsed twice with 2 ml mcilvaine buffer EDTA solution was added to tube and was blended with homogenizer and centrifuged 10min at 2500rpm. Then without transferring any intact tissue supernatant was poured into second 50ml centrifuge tube. After adding 10ml Mcilvaine buffer EDTA solution, the tube was caped and using vortex- mixer, tissue plug resuspended. The suspension was shacked for 10min, centrifuged the suspension was shacked for 10min, centrifuged 10min at 2500rpm and then the supernatant was added to first supernatant in second tube. All the steps were repeated until supernatents from 3 extraction were collected in second tube. The suspension then mixed and centrifuged for 20min at 2500 rpm and supernatant collected.

An SPE cartridge was conditioned with 10 ml of HPLC grade water. The final extract was applied on to cartridge. OTC eluted with 3 ml methanolic oxalic acid solution and diluted 5 ml with HPLC grade water. The tube vortex 30second and 20 µL was injected into HPLC system (Salehzadeh *et al.* 2006) [13].

Chromatographic conditions

The HPLC system (Shimadzu, Japan) equipped with pump, UV detector was used in the study. The chromatographic column was a reversed-phased C18 column. The mobile phase used was 0.03 M oxalic acid, methanol, acetonitrile (60:20:20, v/v/v) by isocratic elution. The flow-rate was 0.5 mL/min, and the UV detector was set at 360 nm. The sample volume injected was 20 µL and the run time was 10 min (Gupta *et al.*, 2014; Ibrahim *et al.*, 2015) [7, 8].

Result and discussion

Table 1: Data of Oxytetracycline residues in broiler chicken meat samples analyzed by HPLC (n = 254)

Type of Tissue	No. of Sample	Positive Samples	Negative Samples	Samples above MRL	Samples below MRL	Positive sample residues concentration range (µg/g)	Approved MRL/MPL (Referring source) (µg/g)
Muscle	68	14	54	5	9	0.048-0.281	0.1, EU 2010
Liver	71	19	52	6	13	0.028-0.285	0.1, EU 2010
Kidney	60	8	52	3	5	0.031-0.370	0.1, EU 2010
Fat	55	7	48	2	5	0.051-0.297	0.1, EU 2010
Total	254	48 (18.89%)	206 (81.1%)	16 (33.33%)	32 (66.66%)		

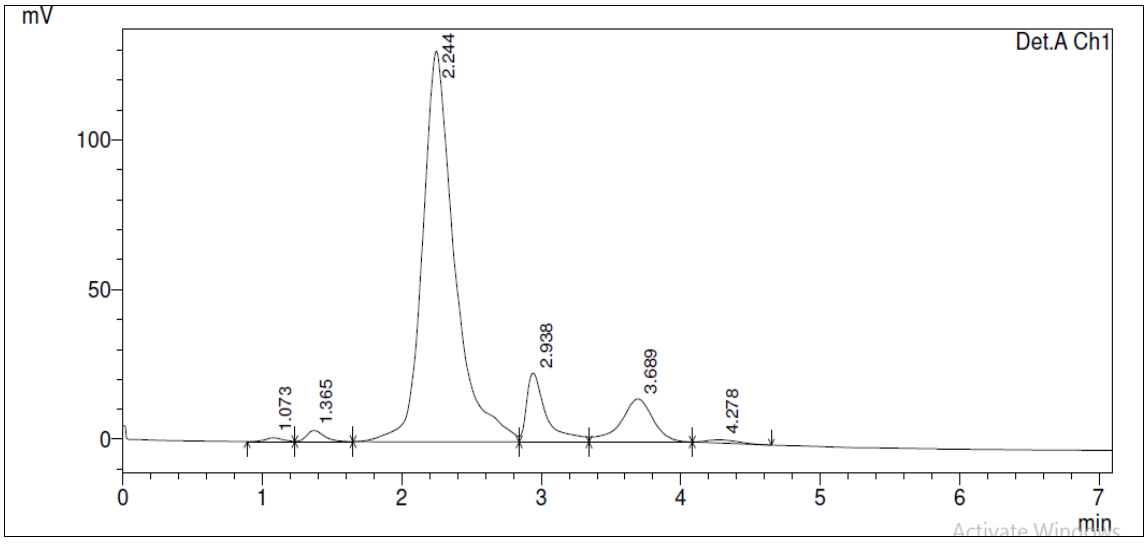

Fig 1: HPLC chromatograms of OTC for a standard solution

The current study was carried out to determine OTC residue poultry meat sample by High Performance Liquid Chromatography (HPLC). A total 452 chicken meat samples (68 samples of muscle, 71 liver, 60 kidney, 55 fat) were collected and drug residual values were analyzed. Later, data was arranged according to the permissible MRL (Maximum Residue Limits). The method for OTC was found to be linear and reproducible in the concentrations ranging 0.028 to 0.370 µg/g. A retention time of 2.23 min was observed. The analysis revealed that 18.89 % meat samples were positive. Out of it, 33.33 % samples were having concentration above the MRL and 66.66 % samples were having residual concentration below the MRL as presented in Tables 1.

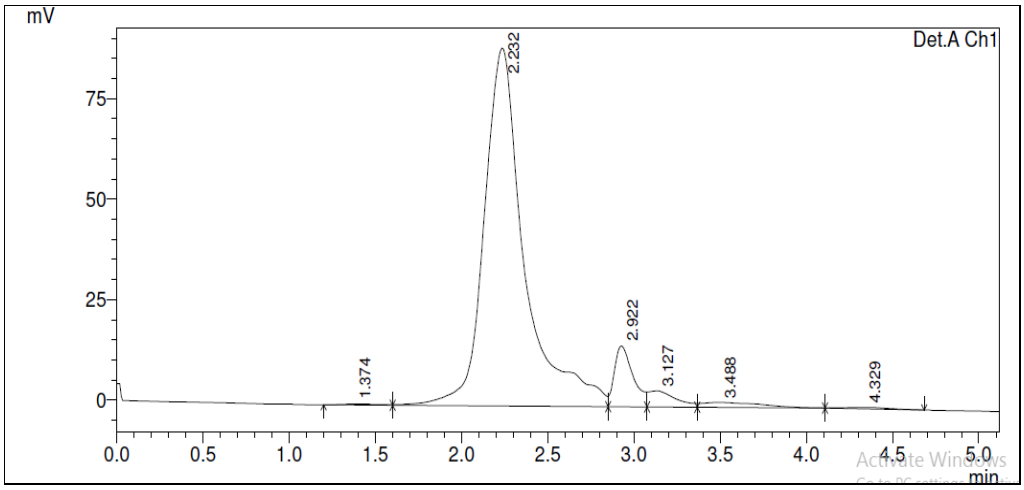

Fig 2: HPLC chromatograms of OTC for a chicken meat sample

Antibiotic compounds in animal products above the MRL are causing severe problems around the world. Antibiotic residues are produced in animals due to a lack of information about medication withdrawal periods and the misuse or overuse of antibiotics. (Seri *et al.*, 2013; Darko *et al.*, 2015) [9, 10]. Many experiments have shown that antimicrobial tolerance can develop in animals as a result of exposure to these agents, and that this resistance can then be passed on to human pathogens (Hoelzer *et al.*, 2017; Yorke and Froc, 2000). Salehzadeh *et al.* (2006) [11, 12, 13] reported that 88.21% of chicken meat samples were positive to antibiotic residues. It is higher than our result. Many searchers were discrepant with our finding. Continuous treatment with antibiotics will results in accumulation of residues in different body parts of animals/poultry. The presence of antibiotics in broiler chicken muscle, liver, kidney, and fat was explored in this research. Endothelial cells in the hepatic sinusoids and peritubular capillaries in the kidney have larger fenestrae (50–150 nm in diameter) that favor the accumulation of drugs in the liver and kidneys (Verma *et al.*, 2020) [14].

Conclusion

The results of our study which are revealing 33.33% samples are above MRL values pose an alarming situation for serious public health concerns to humans and animals, such as toxicity, allergic reactions, and resistance development. It is important to educate poultry farmers and instruct field veterinarians on the principle of withdrawal time and antibiotic judicious use. Because antibiotics are commonly used as feed additives in poultry rations, a withdrawal time should be considered in poultry farms before marketing them for human consumption. Global and international food and drug agencies should also take precautions to ensure that antimicrobials are used responsibly in food-producing animals.

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