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Detection of ciprofloxacin in muscle, liver and kidney of broiler chicken

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Abstract
The present study was undertaken to determine the residues of ciprofloxacin in muscle, liver and kidney of broiler chicken. On the basis of surveillance study, commonly used antibiotic ciprofloxacin was selected for quantifying the concentration in broiler chicken meat samples. The study was conducted from December 2017 to May 2018. The HPLC system was equipped with photodiode array. The results indicated that out of 180 samples analysed, 3 muscle samples of target stations viz. T_2, T_5 and T_8 were found positive for ciprofloxacin residues with concentrations of 0.21, 0.22 and 0.125 µg/kg, respectively. However all concentration values of residues were below MRL as indicated by European Union Codex. Samples collected from other target stations did not show residual concentration of ciprofloxacin in muscle, liver and kidney samples.

Keywords: Surveillance, antibiotic, photodiode array, ciprofloxacin

1. Introduction
The wide spread use of antibiotics in poultry industry resulted in the presence of residues in foodstuffs leading to a potential health hazards for consumers which includes; carcinogenicity, mutagenicity, bone marrow toxicity and allergy [7] as well as appearance of a resistant strains of pathogenic bacteria [5]. Healthy safe food must be free from antibiotics residues or their limits below the maximum residual limits (MRL) recommended by the international Codex Alimentarius Commission (CAC). Accumulation of drug residues in animal tissues poses risks to human health and may result from blind of veterinary drugs without respect to their withdrawal period [9].
Ciprofloxacin is a broad spectrum antibiotic of synthetic fluoroquinolone group. It acts by damaging the bacterial DNA and can enter cells easily. It is often used to treat intracellular pathogens. Ciprofloxacin related to the increased prevalence of resistant bacteria especially campylobacter spp. Chondrotoxic effects and tendon rupture can be induced by fluoroquinolone which emphasis the importance of prevention in children and young people [8]. Owing to their broad spectrum of activity against a wide range of bacteria and their physicochemical properties, fluoroquinolones are not restricted to use as human medicine, but also find wide applications in the treatment and prevention of veterinary diseases in food-producing animals and are even used as growth-promoting agents [10, 12].
The present study was therefore aimed to determine the residues of ciprofloxacin in meat samples of chicken outlets located in and around Jabalpur.

2. Material and Methods
The study was conducted from December 2017 to May 2018

2.1 Collection of meat samples
A total of 180 poultry meat samples including muscle, liver and kidney consisting 60 each were collected from slaughtered poultry of selected targeted area (government and private sector poultry farms) located in and around Jabalpur. Approximately 10 gram of muscle, liver and kidney samples each of the same poultry were aseptically collected and transported to the laboratory in thermo-cooled container jacket with ice and were stored in refrigerator at 4°C till processing.

2.2 Chromatography condition

The (HPLC) unit of High Performance Liquid Chromatography Mass Spectrometer (LCMS-8030, Shimadzu, Japan), consisted of mobile phase reservoir, degasser, HPLC pump, sample injector, gourd column, main column, detector, data collection unit, waste or fraction collector with NEXERA software was used for quantification of ciprofloxacin residues. Chromatographic condition was maintained as described with significant modification. Particle separation was done using hypersil column with C_{18} selectively (Supelco USA, column dimension: 150×2.1 mm, particle size: 1.9 µm) and the temperature of column was set at 30^0C. The mobile phase consisting of 1 ml Ortho phosphoric acid (85 per cent v/v), 100 ml water HPLC grade with acitonitrile HPLC grade, 87:12 (v/v) then pH was adjusted to 2.3 with triethylamine HPLC Grade, The Mobile phase was filtered by 0.22 µ nylon syringe filter before use. Flow rate for the mobile phase was 1ml.min^{-1}. The temperature of column oven was 25±0.5ºC. The effluent was monitored at 278 nm wavelength.

2.3 Chemicals

The chemicals and techniques used for extraction, detection and quantification of residual concentration of ciprofloxacin was : Methanol: HPLC grade, Chromadolv® Sigma-Aldrich, Acetonitrile: HPLC grade, Chromadolv® Sigma-Aldrich, Ortho Phosphoric acid 85 per cent: CDH, Water: HPLC grade, Chromadolv® Sigma-Aldrich, Triethylamine: HPLC grade, Rankem, Trichloro acetic acid AR., 99 per cent: HPLC grade, Hi Media.

2.4 Preparation of standard

Stock standard solution (100 µl/ml): 0.01 g of ciprofloxacin was dissolved in 100 ml of water. The solutions were stored at 4^0C and were stable for at least 1 month. Working standard solution (10 µl/ml): 1ml of 100 µl/ml of ciprofloxacin and stock standard were mixed and diluted to 10 ml with deionised water.

2.5 Samples preparation

The sample preparation or pretreatment procedure involved sample extraction and cleanup processes before analysis by chromatographic technique. In the present study extraction and cleanup procedure for ciprofloxacin in chicken muscle, liver and kidney samples were done as per the method described by Ammar *et al.* [3] with slight modification.

1. Two gram of muscle, liver and kidney were homogenized in mortar and pastel. Placed in a centrifuge tube and 8 ml (5 per cent) trichloroacetic acid (HPLC grade) was added.
2. The sample was vortexed well for homogenization upto1 minute and then centrifuged at 14000 rpm for 5 minutes.
3. The supernatant was filtrated through 0.22 µ nylon filter
4. The 20 µl of filtrate is put in autosampler glass vial having septa and then put into the auto sampler of HPLC apparatus for analysis.

3. Results and Discussion

The mobile phase exhibited good separation of ciprofloxacin from matrix with a mean retention time of 5.16 minutes at wavelength of 278 nm. The limit of detection (LOD) of ciprofloxacin was 0.039 µg/kg and limit of quantification (LOQ) was 0.01 µg/kg. The recovery percentage of ciprofloxacin standard was ranging from 80-94 per cent. Chromatogram exhibited sharp peak and purity at different concentration levels of ciprofloxacin. Ciprofloxacin standard was used in concentrations of 10, 5.0, 2.5, 1.25, 0.625, 0.3125, 0.156 and 0.078 µg.ml^{-1} and their peak area (mAu) was calculated to be 753048, 397433, 222798, 144667, 96973, 69182, 50968 and 38759, respectively.

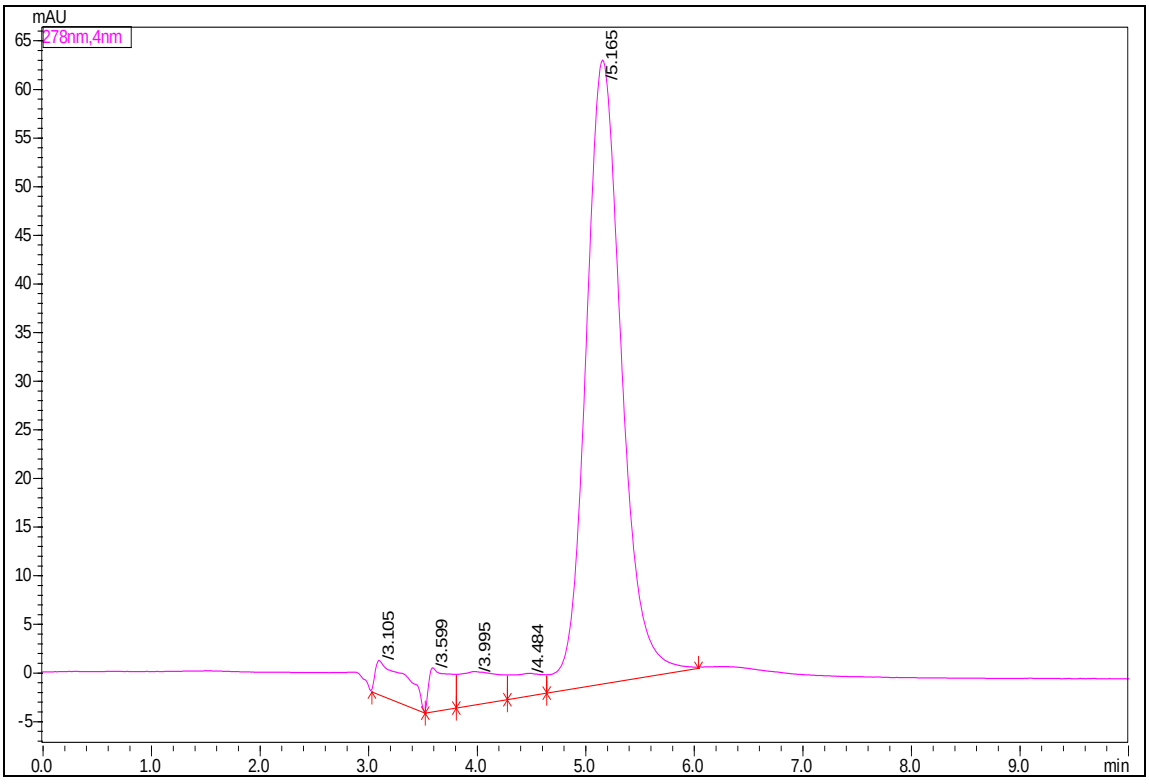

Fig 1: Representative chromatogram of Ciprofloxacin at 5.16 minutes Retention Time

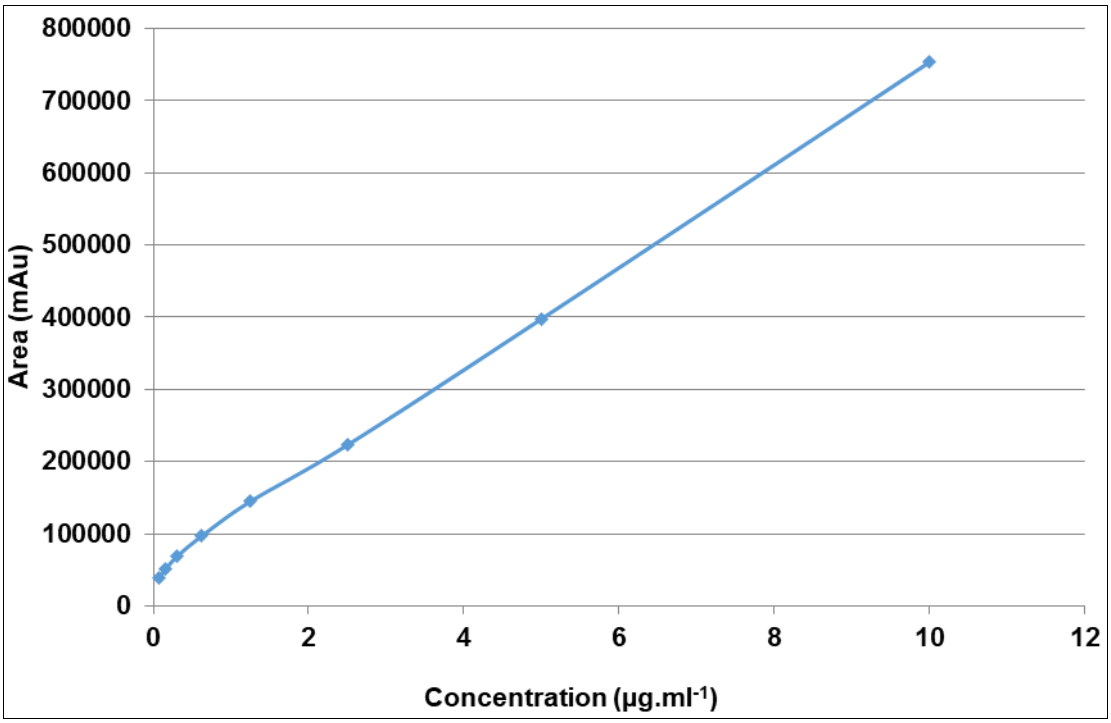

Fig 2: Linear Calibration curve of Ciprofloxacin standard

3.1 Quantification of residual concentration of Ciprofloxacin in muscle, liver and kidney samples of chicken meat using HPLC

A total of 180 chicken meat samples were analysed consisting 18 samples each of muscle, liver and kidney collected from each target stations. Out of 180 samples analysed, 3 muscle samples of target stations, viz. T_2, T_5 and T_8 were found positive for ciprofloxacin residues with concentrations of 0.21, 0.22 and 0.125 µg/kg, respectively. However all concentration values of residues were below MRL as indicated by European Union Codex.

Samples collected from other target stations did not show residual concentration of ciprofloxacin in muscle, liver and kidney samples. Mean residual concentration of ciprofloxacin in muscle, liver and kidney samples of various target stations has been depicted in Table 1.

Table 1: Mean residue concentrations of Ciprofloxacin (µg/kg) in muscle, liver and kidney samples of chicken meat from different target areas.

Target areas	Number of samples	Mean residual concentration (µg/kg)		
		Muscle	Liver	Kidney
T-1	18	ND	ND	ND
T-2	18	**0.21**	ND	ND
T-3	18	ND	ND	ND
T-4	18	ND	ND	ND
T-5	18	**0.22**	ND	ND
T-6	18	ND	ND	ND
T-7	18	ND	ND	ND
T-8	18	**0.12**	ND	ND
T-9	18	ND	ND	ND
T-10	18	ND	ND	ND

ND: Not Detected

Maximum residual limit of ciprofloxacin in chicken meat as: 100µg/kg=100 ppb for muscle, 200 µg/kg=200 ppb for liver, and 300µg/kg=300ppb for kidney recommended by European Union Codex.

The present results clearly showed the presence of ciprofloxacin residues in muscle samples. However, kidney and liver samples did not exhibit residual concentration of ciprofloxacin. The results are in close agreement to the findings of [2] [6] who also reported that muscles had the largest concentration of ciprofloxacin residues than the liver, kidney and skin. However, the present findings differ from the observations of [12] who found that ciprofloxacin residual concentration is more in liver and kidney as compared to muscle tissues of poultry. The detectable residual concentration of ciprofloxacin may vary from lab to lab due to variation the collection time of meat samples and withdrawal period followed by poultry farms. [4] Reported higher concentration of ciprofloxacin residues to the extent of 30.1 µg/kg in chicken meat samples where as in current study the residual concentration of ciprofloxacin was ranging between 0.21 and 0.125 µg/kg. Similarly, [1] detected ciprofloxacin residual concentration as 300 µg/kg in chicken meat.

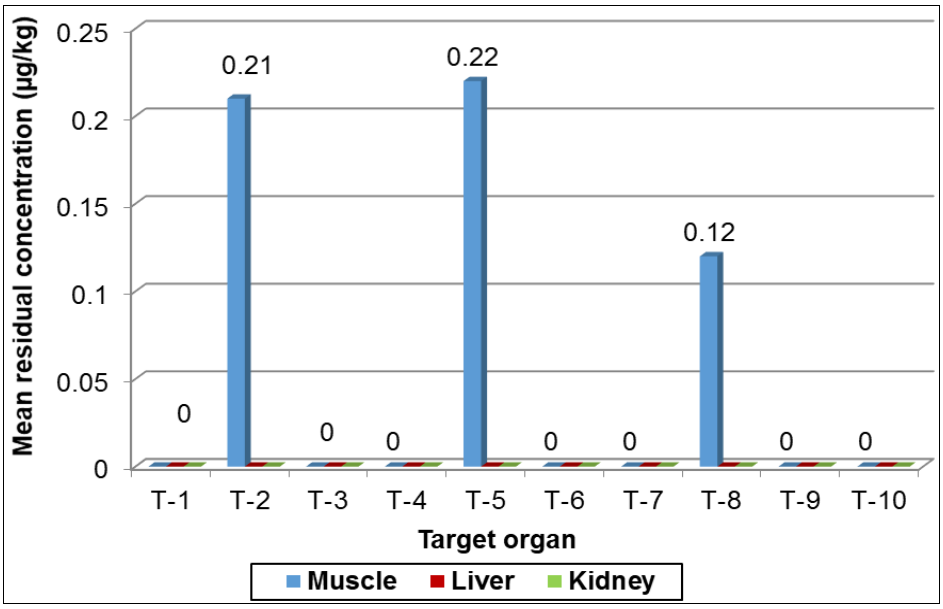

Fig 3: Mean residue concentration of Ciprofloxacin (µg/kg) in muscle, liver and kidney samples of chicken meat from different target areas.

4. Conclusion

On the basis of information gathered from the present study, Out of 180 samples analysed, 3 muscle samples were found positive for residual concentration of ciprofloxacin all positive samples were below MRL. Results confirmed the presence of antibiotic residues of ciprofloxacin in chicken meat which pose a potential hazard to consumers. So, veterinary authorities should control the use of antibiotics in poultry farms and banned their use as growth promoter.

5. Acknowledgement

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6. References

1. Ahmed AM, Gareib MM. Detection of some antibiotics residues in chicken meat and chicken luncheon. Journal of Chemistry Environment Health. 2016; 2(2):315-323.
2. Amjad H, Iqbal J, Naeem M. Analysis of some residual antibiotics in muscle, kidney and liver samples of broiler chicken by various methods. Pakistan Academic Science. 2005; 42(4):223-231.
3. Ammar AM, Abd El-Aziz NK, Hanafy MS, Ibrahim OA. Serotypes profile of avian salmonellae and estimation of antibiotic residues in chicken muscles using high-performance liquid chromatography. Advances in Environmental Biology. 2016; 10(7):173-179.
4. Hasanen FS, Mohammed MM, Mahomud WAH, Hassan MM, Amro FH. Ciprofloxacin residues in chicken and turkey carcasses. Benha Veterinary Medical Journal. 2016; 31(2):136-143.
5. Hussein MA, Khalil S. Screening of some antibiotics and anabolic steroids residues in broiler breast marketed in El-Sharkia governorate. Life Science Journal. 2013; 10(1):2111-2118.
6. Mestorino N, Daniele M, Cardenas MA, Dade M, Errecalde JO. XXII Latin American Poultry Congress; Perfil residual de fosfomicinatrassu administracion oral a pollosparrilleros, 2011.
7. Nisha AR. Antibiotic residues - a global health hazard. Veterinary World. 2008; 1(12):375-377.
8. Petrovi J, Balti M, Upi V, Stefanovi S, Stojanovi D. Residues of enrofloxacin and its main metabolite ciprofloxacin in broiler chickens. Acta *Veterinaria*. 2006; 56(5-6):497-506.
9. Posyniak A, Zmudzki J, Semeniuk S. Effects of the matrix and sample preparation on the determination of fluoroquinolone residues in animal tissues. Journal of Chromatography A. 2001; 914(1-2):89-94.
10. Ramos M, Aranda A, Garcia E, Reuvers T, Hooghuis H. Simple and sensitive determination of five quinolones in food by liquid chromatography with fluorescence detection. Journal of Chromatography B. 2003; 789:373-381.
11. Trouchon T, Lefebvre L. A review of enrofloxacin for veterinary use. Open Journal of Veterinary Medicine. 2016; 6:40-58.
12. Turnidge J. Antibiotic use in animals-prejudices, perceptions and realities. The Journal of Antimicrobial Chemotherapy. 2004; 53:26-27.