Screening of Enrofloxacin and Ciprofloxacin Residues in Chicken Meat by High-Performance Liquid Chromatography

M. K. Verma¹*, A. H. Ahmad¹, Disha Pant¹, Parul Rawat¹, Sonam Sharma¹ and Nidhi Arya¹

¹Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Enrofloxacin and Ciprofloxacin antibiotics are widely used in chicken production for prophylaxis and therapeutics purposes. Existence of these antibiotic residues in chicken meat can pose hazards to human health. 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 enrofloxacin and ciprofloxacin residues in chicken meat samples. The analysis revealed that 43.58% meat samples were positive for enrofloxacin and 38.71% for ciprofloxacin residues. Out of it, 45.17% samples were having concentration above the MRL for enrofloxacin and 50.28% for ciprofloxacin. So it can be concluded that the usage of these antimicrobial in chicken lead contamination of meat and it may cause resistance in consumers and seems to be a public health threat.

Keywords: Enrofloxacin; ciprofloxacin; residues; Uttarakhand.
1. INTRODUCTION

Enrofloxacin and its metabolite ciprofloxacin is belongs from fluoroquinolone class. They are potent synthetic antibiotics that are extensively used in human and veterinary practices [1]. Enrofloxacin is a potent inhibitor of bacterial DNA topoisomerase II (Gyrase) and the DNA Topoisomerase IV which are essential enzymes of cellular processes including DNA replication [2]. These antibiotics have a broad spectrum and high efficacy against pathogens. They are highly effective against Mycoplasma, Gram positive and Gram negative bacteria [3,4]. The indiscriminate use of antimicrobials in food producing animals has resulted in accumulation of residues in their products which is a serious health hazard to the consumers resulting in allergic reactions, imbalance of intestinal microflora and also causes multidrug resistant bacteria [5,6,7].

Significant level of antibiotic residues from animal food products to human may modulate immunological responses in susceptible individual [8]. In order to control this situation, routine quality assurance of food stuff regarding antibiotic residues is warranted. Since, in India, due to extra label use of enrofloxacin for prophylactic and therapeutic purposes, drug residue may be found in poultry meat. Consumption of such meat may act as a potential public health hazard. The present study was aimed to assess the residue levels of these antibiotics 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.

2. MATERIALS AND METHODS

2.1 Chemicals

Enrofloxacin and Ciprofloxacin standard drug were purchased from Sigma Aldrich. Hydrochloric acid (analytical grade), Na₂HPO₄, triethylamine and Acetonitrile (HPLC grade) were purchased from Merck [9].

2.2 Sample Collection

A total 452 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 (July 2017- July 2018).

2.3 Standards and Calibration Curves

The standards for enrofloxacin were made by dissolving 2 mg of pure enrofloxacin in 2 ml of 0.1N-NaOH from which the concentrations of 10, 5, 2.5, 1, 0.5, 0.25, 0.125, 0.1, and 0.01 µg.ml⁻¹ were made in mobile phase (Acetonitrile: 0.05M NaH₂PO₄ (pH 2.5; 35: 65, v/v) and 3.5 mM sodium dodecyl sulphate). The standards for ciprofloxacin were made by directly dissolving 2 mg of pure ciprofloxacin in 2 ml of mobile phase. And further dilutions were made in similar pattern as that for enrofloxacin. 20 µl of these concentrations was injected into HPLC. The standard calibration curve for enrofloxacin and ciprofloxacin was obtained by plotting concentrations verses mean of the peak areas obtained for their respective standards [10].

2.4 Extraction of Samples

Five gram from each sample was weighed and ground in pestle and mortar for several minute. Then, ground sample was transferred in a centrifugation tube and 15 mL of 0.3% metaphosphoric acid: acetonitrile (1:10, v/v) was added followed by homogenization for 3 min. The mixture was sonicated at 200 mA and centrifuged at 20000 rpm for 10 mins. Supernatant was collected in nitrogen evaporator glass tubes with the help of pipette. Then clean up procedure was carried out using solid phase extraction (SPE). Cartridges of SPE were preconditioned with 2.5 ml methanol and 2.5 ml of HPLC grade water prior to filtering the sample. Then mixture was filtered through the 0.45 µm syringe filter (Micropore). 20 µL of the filtered sample was injected into HPLC [11,12].

Mobile phase was acetonitrile: 0.05M NaH₂PO₄ (pH 2.5; 35: 65, v/v) containing 3.5 mM sodium dodecyl sulphate. The flow rate was kept at 0.6 mL.min⁻¹. Chromatography was performed at 22°C with UV detection at 278 nm [13,14]. Standard calibration curve and HPLC chromatograms of enrofloxacin and its metabolite ciprofloxacin for a standard solution and chicken samples are shown in Figs. 1, 2, 3 and 4 respectively.

3. RESULTS

A total 452 chicken meat samples (114 samples of muscle, 113 liver, 108 kidney, 117 fat) were collected and drug residual values were analyzed by using HPLC. Later, data was arranged according to the permissible MRL (Maximum
Residue Limits). The limit of quantification (LOQ) for enrofloxacin and ciprofloxacin was 0.0125 µg.ml⁻¹. The method for enrofloxacin was found to be linear and reproducible in the concentrations ranging 0.0125 to 10 µg.ml⁻¹. A retention time of 7.37 min for enrofloxacin and 9.95 min for ciprofloxacin was observed. The analysis revealed that 43.58% meat samples were positive for enrofloxacin and 38.71% for ciprofloxacin residues. Out of it, 19.69% samples were having concentration above the MRL for enrofloxacin and 19.91% for ciprofloxacin and 23.89% samples were having residual concentration below the MRL for enrofloxacin and 18.80% for ciprofloxacin as presented in Tables 1 and 2.

![Fig. 1. Standard calibration curve of enrofloxacin](image)

![Fig. 2. Standard calibration curve of ciprofloxacin](image)

Table 1. Data of enrofloxacin residues in broiler chicken samples analyzed by HPLC (n = 452)

| 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        | 114           | 43               | 71               | 18                | 25                | 0.048-0.284                                   | 0.1, EU 2010                             |
| Liver         | 113           | 63               | 50               | 29                | 34                | 0.028-0.284                                   | 0.2, EU 2010                             |
| Kidney        | 108           | 58               | 50               | 26                | 32                | 0.031-0.370                                   | 0.3, EU 2010                             |
| Fat           | 117           | 33               | 84               | 16                | 17                | 0.031-0.188                                   | 0.1, EU 2010                             |
| Total         | 452           | 197              | 255              | 89                | 108               |                                               |                                           |
|               | (43.58%)      | (56.41%)         | (19.69%)         | (23.89%)          |                   |                                               |                                           |
Fig. 3. HPLC chromatograms of enrofloxacin and its metabolite ciprofloxacin for a standard solution

Fig. 4. HPLC chromatograms of enrofloxacin and its metabolite ciprofloxacin for a chicken meat sample

Table 2. Data of ciprofloxacin residues in broiler chicken samples analyzed by HPLC (n = 452)

| 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        | 114           | 37               | 77               | 17                | 20               | 0.031-0.175                                    | 0.1, EU 2010                             |
| Liver         | 113           | 57               | 56               | 34                | 23               | 0.014-0.247                                    | 0.2, EU 2010                             |
| Kidney        | 108           | 52               | 56               | 28                | 24               | 0.058-0.368                                    | 0.3, EU 2010                             |
| Fat           | 117           | 29               | 88               | 11                | 18               | 0.017-0.121                                    | 0.1, EU 2010                             |
| Total         | 452           | 175              | 277              | 90                | 85               | (38.71%) (61.28%) (19.91%) (18.80%)            |                                          |

4. DISCUSSION

The presence of antibiotic residues in animal products above MRL in food-producing animals has serious problems worldwide. Lack of knowledge about the drug withdrawal time and the misuse or overuse of antibiotics lead the formation of antibiotics residues in the animal [15,16]. Many reports indicated that antimicrobial resistance may arise as a result of exposure in animals to these agents and that the resistance may be transferred to human pathogens organisms [17,18]. In the present work we examined broiler chicken muscle, liver, kidney and fat for the presence of enrofloxacin and ciprofloxacin residues. The results showed that 43.58% and 38.71% meat samples were positive for enrofloxacin and ciprofloxacin respectively. Our result were consistent with Buket et al. [19] in Turkey mentioned that one hundred eighteen
sample were examined and found 45.7% of chicken meat samples. Amro et al. [20] in Egypt, revealed that the incidence percentage were 40% in chicken meat. Chaiba et al. [21] reported that 36.15% of chicken meat samples were positive to antibiotic residues. It is slightly less than our result. Many searches were discrepant with our finding. Both antibiotics are lipophilic in nature, they are distributed into various body tissues, including lungs, liver, kidney, muscles and skin. Further, a longer half-life favours their longer persistence in tissues [22]. In our study, liver and kidney samples were found to be more positive as compared to muscles and fat samples which could be the result of metabolism of these drugs in liver. Further, 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 [23,24,25].

5. CONCLUSION

The results of our study which are revealing 19.69% for enrofloxacin and 19.91% for ciprofloxacin samples are above MRL values pose an alarming situation. There is an utter need to educate the poultry farmers and to train field veterinarians about the concept of withdrawal period and judicious use of antibiotics. Antibiotics are common feed additives used in poultry ration so withdrawal period should be taken in consideration in poultry farms before selling them out for human consumption. National and international food and drug authorities should also adopt judicious approaches to ensure prudent use of antimicrobial in food producing animals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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