Screening of Enrofloxacin and Ciprofloxacin Residues in chicken meat by High-Performance Liquid Chromatography

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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.

Key words

Enrofloxacin, Ciprofloxacin, Residues, Uttarakhand.

Introduction

Enrofloxacin and its metabolite ciprofloxacinis belongs from fluoroquinolone class.They are potent synthetic antibiotics that are extensively used in human and veterinary practices **(Lopez *et al.*, 2015)**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**(Trouchon and Lefebvre, 2016).**These antibiotics have a broad spectrum and high efficacy against pathogens. They are highly effective against *Mycoplasma*, Gram positive and Gram negative bacteria **(Ding *et al.*, 2020; Hooper *et al.*, 2001).** 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 mutidrug resistant bacteria **(Lewis and Cook 2014; CVMP 2007;Alewy *et al.*, 2018).**Significant level of antibiotic residues from animal food products to human may modulate immunological responses in susceptible individual **(Ramatla *et al.*, 2017).** 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 enrofloxacinfor 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.

Materials and Methods

Chemicals:

Enrofloxacin and Ciprofloxacin standard drug were purchased from Sigma Aldrich. Hydrochloric acid (analytical grade), Na_2HPO_4, triethylamine and Acetonitrile (HPLC grade) were purchased from Merck **(Patyra & Kwiatek, 2017).**

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).

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 0.01µg.ml^{-1} were made in mobile phase(Acetonitrile: 0.05M NaH_2PO_4(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. (**Varma *et al.*, 2006**

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 200mA and centrifuged at 20000 rpm for 10mins. 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 2.5ml of HPLC grade water prior to filtering the sample. Then mixture was filtered through the 0.45 µm syringe filter (Micropore). 20µL of the filtered sample was injected into HPLC**(Shu *et al.*, 2002; Moyo and Tavengva, 2019)**

Mobile phase was acetonitrile: 0.05M NaH_2PO_4 (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 22^0 C with UV detection at 278 nm **(Naeem *et al.*, 2006; Qiao and Sun**, **2010).** 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.

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 $\mu g.ml^{-1}$. The method for enrofloxacin was found to be linear and reproducible in the concentrations ranging 0.0125 to 10 $\mu g.ml^{-1}$. 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 Table 1 and Table2.

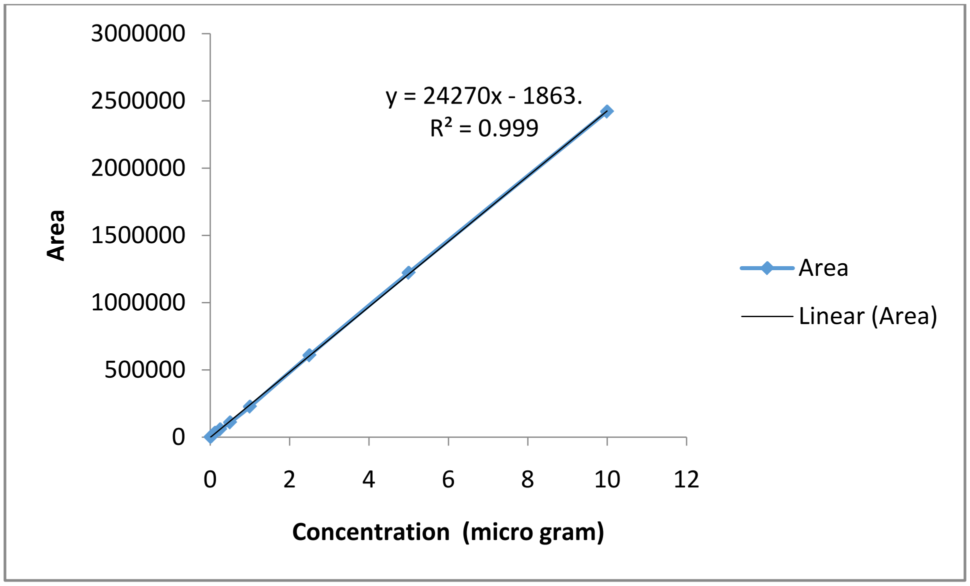

Fig. 1- Standard calibration curve of enrofloxacin

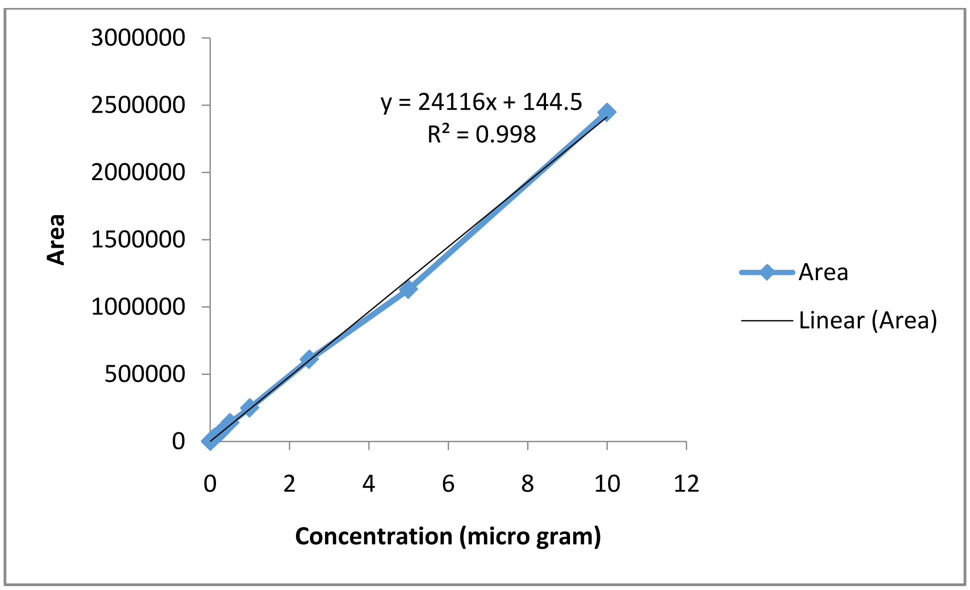

Fig. 2- Standard calibration curve of ciprofloxacin

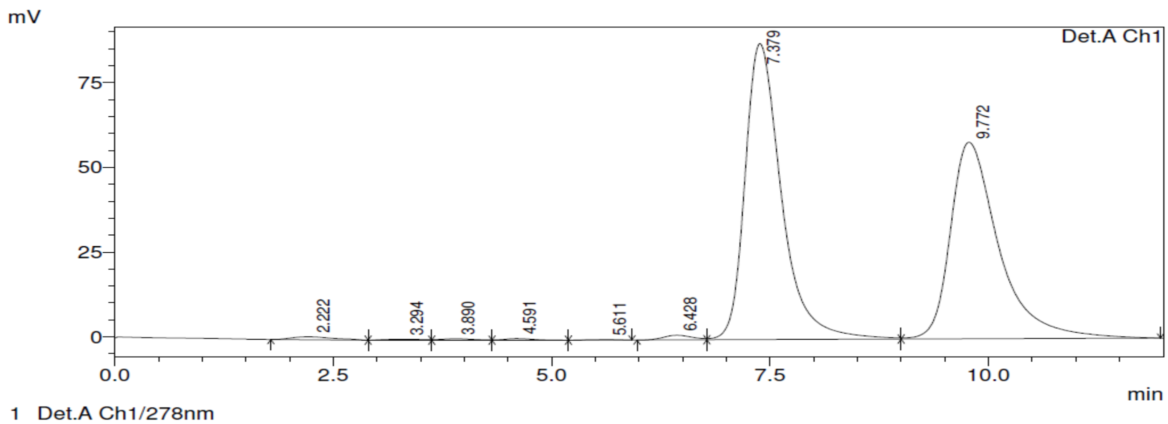

Fig. 3HPLC chromatograms of enrofloxacin and its metabolite ciprofloxacin for a standard solution

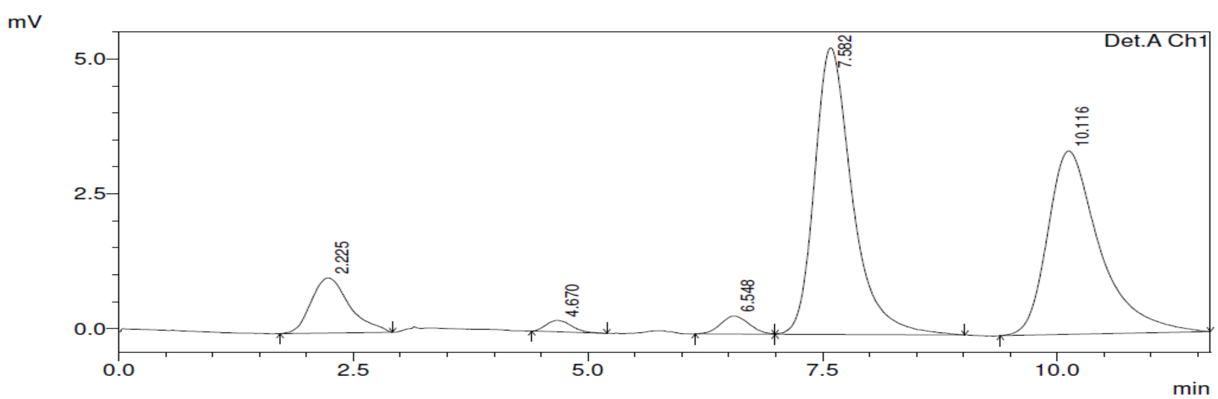

Fig. 4: HPLC chromatograms of enrofloxacin and its metabolite ciprofloxacin for a chicken meat sample

Table 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 (43.58%)	255 (56.41%)	89 (19.69%)	108 (23.89%)		

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 (38.71%)	277 (61.28%)	90 (19.91%)	85 (18.80%)		

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 **(Seri *et al.,* 2013; Darko *et al.,* 2015).** 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 **(Hoelzer et al., 2017;Yorke and Froc, 2000).** 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.*,2013** in Turkey mentioned that one hundred eighteen sample were examined and found 45.7% of chicken meat samples. **Amro *etal.*, 2013** in Egypt,revealed that the incidence percentage were 40% in chicken meat. **Chaiba *et al.*, 2017**reported that 36.15% of chicken meat samples were positive to antibiotic residues. It is slightly less than our result.Many searchers 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 **(Shareef *et al.*, 2009).** 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 **(Aslam *et al.*, 2016; Gregus, 2013;Demetris *et al.*, 2016)**

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.

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