WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 1579-1588. Research Article ISSN 2277– 7105

ESTIMATION OF WITHDRAWAL PERIOD OF CIPROFLOXACIN IN LIVER AND BREAST MUSCLE OF DUAL PURPOSE CHICKEN

Sunil Chandra U.*, Shridhar N.B., Jagadeesh S., Sanganal, Narayanaswamy H.D., Ansar Kamran C. and Ramachandra S.G.

Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bengaluru. Karnataka. 560024.

Article Received on
31 July 2015,

Revised on 20 Aug 2015,
Accepted on 09 Sep 2015

***Correspondence for Author**
Sunil Chandra U.
Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bengaluru. Karnataka. 560024.

ABSTRACT

The study was carried out to evaluate the withdrawal period of ciprofloxacin in liver and breast muscle tissues of dual purpose chicken after oral administration for five days. Indian Rock -3, a dual purpose chicken strain of White Plymoth Rock developed by Karnataka Veterinary Animal Fisheries Sciences University, Bidar was used for the study. Birds in the age group of thirty days, were divided in to two groups. Group I (n=10) birds served as control, did not receive any drug. The tissue samples of these were used for the standardization of the analytical instrument. Group- II (n=80) birds received ciprofloxacin at 8mg per kg body weight orally for five days. Residue analysis in liver and breast muscle were analysed by liquid chromatography-mass spectrometry (LC-MS). The residue concentration of ciprofloxacin was gradually decreased in chicken liver and breast muscle samples starting from day one to day ten after the last dose. Ciprofloxacin was estimated to have a pre slaughter withdrawal period of four to five days in comparison with maximum residue level of 200, and 100 µg / kg for liver and breast muscle. respectively as per European economic community council regulations..

KEYWORDS: ciprofloxacin, LC- MS, liver, breast muscle, maximum residue limit.

INTRODUCTION

Fluoroquinolones are a series of synthetic antibacterial agents that are used in the treatment of a variety of bacterial infections. These agents act by inhibiting the DNA gyrase, thus interfering with the DNA-rejoining reaction and the inhibition of the resealing leading to the

liberation of fragments that are subsequently destroyed by the bacterial exonucleases. Fluoroquinolones are active against some gram-negative bacteria, including *E. coli*, *Enterobacter* species, *Klebsiella* species, *Pasteurella* species, *Proteus* species, and *Salmonella* species, including activity against some gram-positive bacteria, chlamydia, mycobacteria, and mycoplasma. In some regions, the use of fluoroquinolones is approved for the treatment of colibacillosis of chickens and turkeys, fowl cholera in turkeys, and bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus*, and other susceptible organisms. Fluoroquinolones are frequently used in poultry production and human medicine with safety criteria, including withdrawal periods, doses, and treatment duration, as their misuse and abuse may cause bacterial resistance and presence of residues in edible tissues. Consequently, the consumption of animal products with fluoroquinolone residues may result in transmission of resistant bacteria.[1]

Ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro- 4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid], is a fluorinated quinolone with a very broad antimicrobial spectrum and high bactericidal activity. Ciprofloxacin has high activity against a broad spectrum of bacteria, i.e. gram-negative bacteria including various species of *Enterobacteriaceae* and *Pseudomonas,* some gram-positive bacteria, and many aerobic and facultatively anaerobic bacteria, *Mycoplasma* and *Rickettsia*. As compared with the developed fluorine-containing pyridonecarboxylic acid derivatives, i.e. norfloxacin, ofloxacin, pefloxacin and enoxacin, ciprofloxacin has activity about four times greater against almost all of the above bacteria. In vitro evaluation of various quinolone antibacterial agents against selected veterinary bacterial pathogens showed that ciprofloxacin was the most active quinolone particularly against gram-negative bacteria, and mycoplasmal species.[2] In poultry, ciprofloxacin is often recommended for respiratory tract infections, gastrointestinal tract infections and urinary tract infections caused by *Campylobacter, E. coliI, Haemophilus, Mycoplasma*, *Pasteurella* and *Salmonella* species.[3]

Withdrawal Time (WDT) is the period of time required after completion of treatment needed for tissue concentrations of the drug and/or its metabolites to deplete to less than the established Maximum Residue Limits (MRLs). The administration of drugs to food-producing animals without an adequate WDT might lead to excessive concentrations of residues in foods intended for human consumption, representing a risk to public health, including stimulation of bacterial resistance, alterations on intestinal microflora and

hypersensitivity reactions. The ever increasing use of fluoroquinolones in poultry industry has caused their residual deposition in the poultry products resulting in the drug resistant bacteria. It has become a matter of foremost importance to screen the poultry birds of these residual antibiotics, down to the safer MRL. The administration of fluoroquinolones to food-producing animals without an adequate withdrawal period might result in violative concentrations of residues in foods destined for human consumption posing a risk to public health, including stimulation of bacterial resistance, alterations on intestinal microflora and hypersensivity reactions. This fact marked the importance to develop new methods for a fast, simple and accurate quantification of residues of the antibacterials in food producing animals. Various analytical techniques such as atomic absorption, spectrometry, polarography, differential pulse polarography, capillary zone electrophoresis, spectrofluorometry and HPLC, liquid-liquid extraction, Solid phase extraction by LC- MS/MS and Nuclear Magnetic Resonance (NMR) were used to extract metabolites and determination of antibiotic residue concentration in the animal tissues.[4] LC-MS/MS provides superior specificity and sensitivity and can be used to develop highly accurate and reproducible assays. The primary advantage LC-MS has that it is capable of analysing a much wider range of compounds that are thermally labile, exhibit high polarity or have a high molecular mass.

Indian Rock-3 (IR-3) is a dual purpose chicken strain of White Plymouth Rock developed by Karnataka Veterinary Animal and Fisheries Sciences University, Bidar. It was essential to generate tissue depletion data in order to arrive at conclusion regarding MRLs and withdrawal period for ciprofloxacin in dual purpose chicken. In view of the marked species variation in the data of antimicrobial drugs, present study was planned to determine the withdrawal period of ciprofloxacin in liver and breast muscle tissues of dual purpose chicken. Following oral administration at the dose of 8 mg per kg body weight for five days in the dual purpose chicken.

MATERIALS AND METHODS

The study was conducted in dual purpose chicken : Indian Rock -3 (IR-3), a strain of White Plymoth Rock developed by KVAFSU, Bidar, The study was performed in animal house at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru. Healthy birds aged thirty days old (n=90) were selected for the study.

Drugs

Ciprofloxacin and indomethacin technical grade powder were obtained from Varsha Labs, Bengaluru, India and Sigma Aldrich, (Poole, UK) respectively. HPLC grade formic acid, acetic acid, methanol and acetonitrile were procured from E-merck (Germany). HPLC grade water prepared in house using a Millipore Direct-QTM 5Water System (Millipore, Watford, UK). Filtration of HPLC mobile phase was performed using Sartorius membrane filters [0.45µm] obtained from Sartorius (Epsom, UK) and Solid Phase Extraction cartridges (Orochem Company). Blank tissue samples for the preparation of calibration standards and quality control samples were collected from group- I (n=10) birds and were stored at 20±5°C until analysis.

Experimental animals

For residues analysis and withdrawal time calculation, group-II birds (n=80) were kept under observation for one week prior to commencement of experiment and subjected to clinical examination in order to exclude the possibility of disease. The birds were provided antibiotic-free standard broiler ration for fourteen days. The animal house was maintained at room temperature (25 ± 2^0C) and at 45 to 65 per cent relative humidity. Food and water were supplied ad libitum with standard management practices to keep the birds free from stress. The experimental protocol was approved by the Institutional Animal Ethics Committee.The antibiotic residue analysis was conducted as per European Commission Council Regulation[5].

Ciprofloxacin was administered to group-II birds at the dose of 8 mg per kg body weight orally for five days. The birds were euthanized and immediate exsanguinations on day 1,2,3,4, 5,6,7, 8.9 and 10 after the administration of the last dose of ciprofloxacin (n=8/day). Samples of liver and breast muscle were collected and the tissue samples were stored at -45^0C until assayed for concentrations of ciprofloxacin. The samples were subjected to residual analysis using Liquid Chromatography tandem Mass Spectroscopy (LC-MS).

Based on the the residual concentration, the withdrawal time of ciprofloxacin in dual purpose chicken were established. MRLs calculated from ciprofloxacin area at each sampling time were considered for the determination of withdrawal time in liver and breast muscle, adopting European Agency for the Evaluation of Medicinal Products recommendations.[6]

Experimental conditions

The chromatography was carried out with LC-MS/MS (Agilent Technologies, Waldbron, Germany) Agilent 1200 RRLC system with a solvent delivery pump, auto-degasser, auto sampler and column oven. Electrospray mass spectrometry (ESI-MS) was carried out using a 3200 Q TRAP triple-quadrupole LC-MS/MS system (Applied Biosystems/MDS Sciex), coupled with a Turbo Ion Spray (TISP) source with ESI mode. Applied Biosystems Sciex Analyst software version 1.5 was employed for data acquisition and processing. The separation was performed on a Thermo Scientific BDS Hypersil C18 RP, 100x4.6 mm, 5 µm. Separation was achieved using a gradient elution with the flow rate of 0.7 ml/min, while the injection volume was 20 µl.

Preparation of calibration standard solutions and quality control stocks

Primary stock solution of ciprofloxacin (CIP) for calibration standard and quality control (QC) samples were prepared in methanol. From the primary stock solution, appropriate dilutions were made using Methanol: water (50:50 % v/v) as a diluent to produce working standard solutions of 2000.000, 4000.000, 10000.000, 20000.000, 40000.000, 80000.000, 120000.000, 160000.000 and 200000.000ng/mL. These solutions were used to prepare relevant calibration curve (CC) standards. Another set of working solutions of ciprofloxacin was prepared in the diluent (from primary stock) at concentrations of 2000.000, 6000.000, 100000.000 and 180000.000ng/mL respectively for QC samples (LLOQC, LQC, MQC & HQC). The calibration standards and quality control samples were prepared by spiking 0.010mL of the spiking stock solution (Ciprofloxacin) into 0.190mL of screened blank chicken plasma. Calibration samples were made at concentrations of 100.000, 200.000, 500.000, 1000.000, 2000.000, 4000.000, 6000.000, 8000.000 and 10000.000ng/mL. Quality control samples were prepared at concentrations of : 100.000ng/mL for Lower limit of quality control (LLOQC); 600.000ng/mL for Lower quality control (LQC); 5000.000ng/mL for Medium quality control (MQC) and 9000.000ng/mL for Higher quality control (HQC).

RESULTS AND DISCUSSION

In the present study, residual concentrations of ciprofloxacin in liver and breast muscle tissues of dual purpose chicken were analysed by LC-MS/MS after oral administration of ciprofloxacin at 8 mg per kg body weight for five consecutive days. LC- MS/MS method was selected because of its high specificity and accuracy. Applied Biosystems Sciex Analyst software version 1.5 was employed for data acquisition and processing. The separation was

performed on a Thermo Scientific BDS Hypersil C8 RP, 100x4.6 mm, 5 µm. Separation was achieved using a gradient elution with the flow rate of 0.7 ml/min, while the injection volume was 20 µl.LC-MS-MS has been used for similar purpose by other workers.[7,8]

Liver

High residue level of ciprofloxacin was observed on day one and gradually decreased up to tenth day in liver tissue samples after the last dose of administration. Highest ciprofloxacin residue concentration detectable in liver was 1321.85 ± 0.91µg per kg and the residue concentration was 59,83 ± 0.28µg per kg on day ten after after the last dose of ciprofloxacin. The concentration of ciprofloxacin on day four was 189.22 ± 0.98µg/kg., which was less than the Maximum Residual Level (MRL) fixed for fluroquinolones (200 µg/kg) in liver tissue. Thus the withdrawal period for ciprofloxacin in liver of dual purpose chicken was estimated to be four days

Jelena *et al.*[9] reported high residue levels of enrofloxacin and ciprofloxacin in liver tissue on day one at 1196.1 µg per kg and 187 µg per kg respectively, compared to other tissues and decreasing in the enrofloxacin residue concentration up to ninth day was 24.8 µg per kg after the treatment. Lim *et al.*[10] reported that high residue concentration of norfloxacin observed in liver tissue was 990 µg per kg on day one compared to other tissues and there was decreased in ciprofloxacin residue concentration up to 70 µg per kg on fifth day after the treatment. Anadon *et al.*[11,12] reported that high residue concentration of difloxacin and its metabolite sarafloxacin in liver tissue were 368.1±52 .5 µg per kg and 10.4±1.2 µg per kg respectively on day 1 compared to other tissues and decreased in difloxacin residue concentration up to fifth day after administration of the final dose of difloxacin.

Breast Muscle

Highest concentration of ciprofloxacin in breast muscle tissue was 446 ± 0.92µg per kg on day one and decreased in residue concentration up to 14.51 ± 0.77µg per kg on day ten after the administration of the last dose of ciprofloxacin in dual purpose chicken. Persistence of ciprofloxacin in animal tissues is attributed to the liphophilicity and tissue perfusion rate of the drug.[13,14]

Withdrawal period was calculated based on the residual concentration of ciprofloxacin in tissues of dual purpose chicken. The MRLs were 200 and 100 µg per kg for liver and breast muscles respectively. Jelena *et al.*[9] reported the withdrawal period of four days for

enrofloxacin in muscle and liver in broiler chicken. Petrovic *et al.*[15] reported a withdrawal period of four days for enrofloxacin and its metabolite ciprofloxacin residues to decrease to an acceptable level in the meat and liver of the broiler chicken. It was concluded that ciprofloxacin was estimated to have four days of withdrawal period in liver and five days of withdrawal period in breast muscle of dual purpose chicken.

Table 1: Intra- and inter-day assay coefficient of variation (CV%) and nominal concentration (%) of ciprofloxacin for reside analysis in liver and breast muscle tissue of dual purpose chicken.

Concentration (ng/ml)	Intra-day assay (n= 8)			Inter-day assay (n= 8)		
	Mean ±SD (ng/ml)	CV (%)	Mean concentration (%)	Mean ±SD (ng/ml)	CV (%)	Mean concentration (%)
100 (LLQC)	98.86±0.30	10.65	98.76	97.98.46±0.26	11.46	92.84
300 (LQC)	291.68±0.12	12.08	97.23	261.46±0.13	13.41	87.15
5000 (MQC)	4425.78±0.20	6.87	88.52	4671.08±0.21	7.77	93.42
9000 (HQC)	8206.93±6.16	6.61	91.19	7315.38±7.04	7.65	81.28

*LLOC: Lower limit of quality control.

LQC: Lower quality control.

MQC: Medium quality control.

HQC: High quality control.

Table 2: **Concentration (µg per kg) of ciprofloxacin in dual purpose chicken tissues. Values are expressed as Mean ± SE; n=8 per day.**

Days	Liver	Breast muscle
1	1321.85 ± 0.91	446 ± 0.92
2	594.73± 1.33	228.03 ± 0.62
3	364.03 ± 0.76	149.23 ± 0.71
4	189.22 ± 0.98	108.87 ± 0.76
5	137.56 ± 1.01	82.33 ± 0.62
6	111.71 ± 0.74	50.83 ± 0.74
7	109.68 ± 0.81	39.53 ± 0.56
8	89.76 ± 0.65	28.41± 0.94
9	73.77 ± 0.57	21.29 ± 0.89
10	59,83 ± 0.28	14.51 ± 0.77

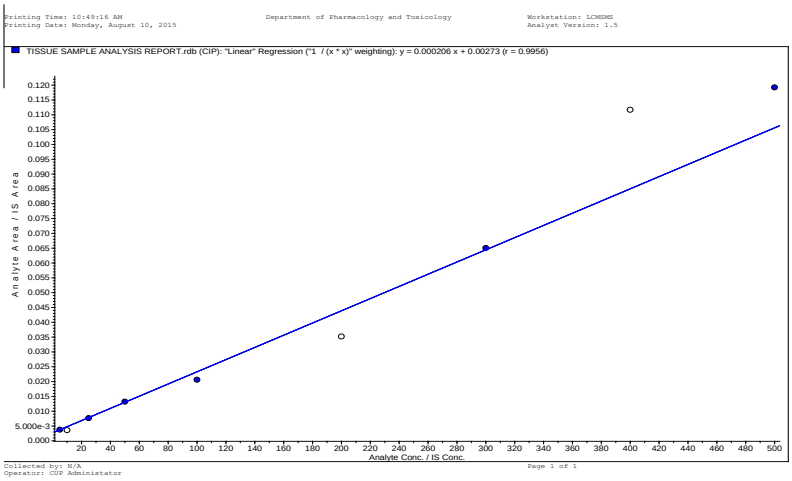

Figure 1: Standard linearity curve for ciprofloxacin extracted blank tissue homogenate sample.

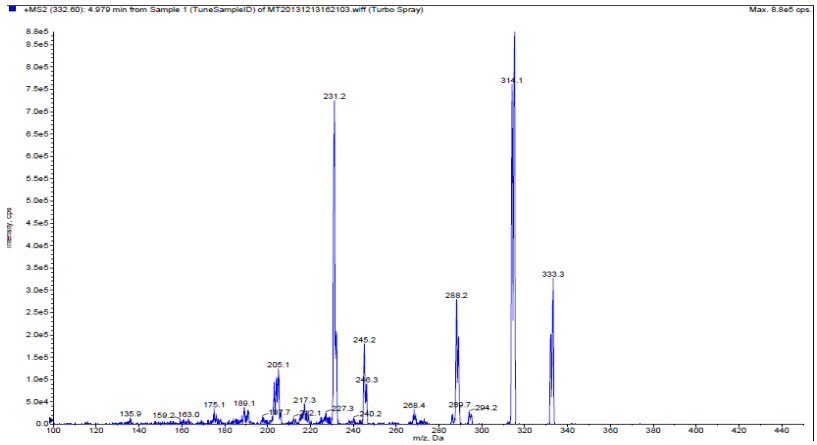

Figure 2: The product ion mass spectra of ciprofloxacin.

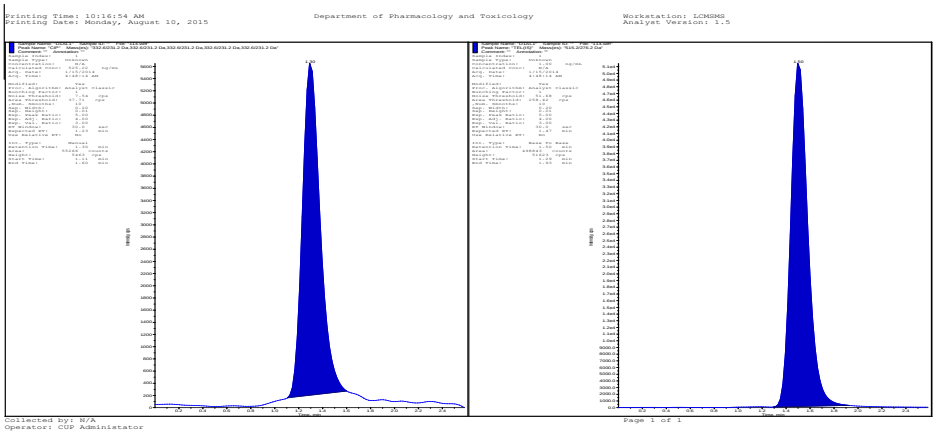

Figure 3: A representative chromatogram for the extracted and homogenized liver tissue on Day 1 after the last dose of ciprofloxacin.

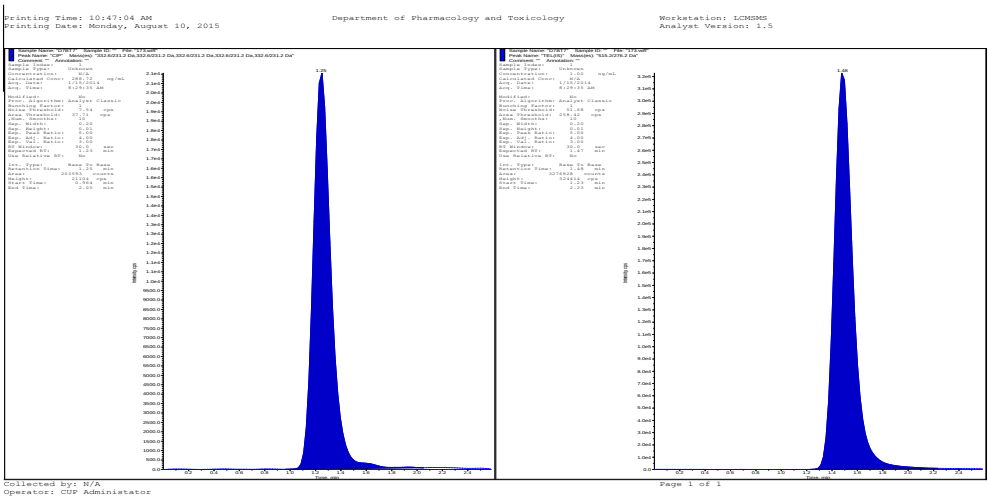

Figure 4: A representative chromatogram for the extracted and homogenized breast muscle sample on Day 1 after the last dose of ciprofloxacin.

REFERENCES

1. Gouvea, R., Santos, F. D., Aquino, M. D. and Pereira, V. D., Fluoroquinolones in industrial poultry production, bacterial resistance and food residues: Brazilian J. Poult. Sci., 2015; 17(1): 1-10.
2. Dimitrova, D.J., Lashev, L.D., Yanev, S.G. and Pandova, V.T., Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin in male and female turkeys following intravenous and oral administration. Vet. Res. Commun., 2006; 30: 415-422.
3. Anadon, A., Martinez, M. R., Larranaga, J., Iturbe, M. A., Martianez, M. J., Diaaz, M. T., Frejo, M. and Martianez., Pharmacokinetics and residues of ciprofloxacin and its metabolites in broiler chickens. Res. Vet. Sci., 2001; 71: 101-109.
4. Amjad, H., Iqbal, J. and Naeem, M., Analysis of some residual antibiotics in muscle, kidney and liver samples of broiler chicken by various methods. Pro. Pakistan. Acad. Sci., 2005; 42(4): 223-231.
5. EEC, 1994. European Commission Council regulation. Laying down a community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, amending regulation no. 1430/94 of 22 June 1994. Off. J. Eur. Comm., 156: 23.
6. EMEA, 1995. The European Agency for the Evaluation of Medicinal Products. Note for Guidance: Approach towards harmonization of withdrawal periods. EMEA/CVMP/036/95, 1-37.

7. Aravind, K.A., Anu, R., Ragvendra, R., Atul, P., Rajesh, M. and Garg, S. K., Pharmacokinetics of levofloxacin following intravenous and intramuscular administartion in cattle calves., Asian. J. Anim. Vet. Adv., 2012; 7(10): 1006-1013.
8. Haritova, A.M., Rusenova. N.V., Parvanov, P.R., Lashev, L.D and Gremmels, J.K., Integration of pharmacokinetic and pharmacodynamic indices of marbofloxacin in turkeys. Antimicrob Agents. Chemother., 2006; 50(11): 3779-3785.
9. Jelena, P., Baltic, M., Cupic, V. and Stefanovic, D., Residues of enrofloxacin and its main metabolite ciprofloxacin in broiler chickens. Acta Veterinaria, 2006; 56(5): 497-506.
10. Lim, J., Parl, B. and Yun, H., Sensitive liquid chromatographic - mass spectrometric assay for norfloxacin in poultry tissue. J. Chromatogr., 2002; 77(2): 185-189.
11. Anadon, A., Martinez, L., Diaz, M. J., Bringas, P. and Martinez, M. A., Pharmacokinetics and residues of enrofloxacin in chickens, Am. J. Vet. Res., 1995; 56(4): 501-504.
12. Anadon, A., Suarez, F., Martinez, M.A., Castellano, V. and Ares, I., Plasma disposition and tissue depletion of difloxacin and its metabolite sarafloxacin in the food producing animals, chickens for fattening. Food chem. Toxicol, 2011; 49(2): 441- 449.
13. Amjad, H., IqbaL, J. and Naeem, M., Analysis of some residual antibiotics in muscle, kidney and liver samples of broiler chicken by various methods. Pro. Pakistan. Acad. Sci., 2005; 42(4): 223-231.
14. Goetting, V., Lee, K. A. and Tell, L.A., Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. J.Vet. Pharmacol.Therap., 2011; 34(6): 521-556.
15. Petrovic, J., Baltic, M., Cupic, V., Stefanovic, S. and Stojanovic D., Residues of enrofloxacin and its main metabolite ciprofloxacin in broiler chickens. Acta Veterinaria, 2006; 56: 5-6. 497-506.