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Pharmacokinetics of levofloxacin through oral route of administration in dual purpose chicken by the LCMS/MS analytical technique

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Abstract

Levofloxacin, a third-generation fluoroquinolone, is the S-isomer of ofloxacin and possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria (North *et al.*, 1998). The study was carried out to evaluate the pharmacokinetic parameters following single oral dose administration of levofloxacin in dual purpose chicken. The study was conducted in 30 to 40 day old healthy dual purpose chicken Indian Rock -3 (IR-3), a strain of White Plymouth Rock developed by Karnataka Veterinary Animal Fisheries Sciences University, Bidar. The study was performed at the department of Poultry Science, Veterinary College, Hebbal, Bengaluru. In the pharmacokinetic study, experimental birds were (n= 10) were administered with levofloxacin at 10 mg/ kg bw single dose through oral route directly in to crop using a thin plastic tube attached to a syringe. Following oral administration, blood samples were collected at time 0 min, 5 min, 10 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 16 h and 24 h. Plasma was separated soon after blood collection by centrifugation (8000 rpm at 4 °C for 10 min), stored at -20 °C until the analysis of the pharmacokinetic parameters. The determination of the pharmacokinetic parameters like AUC₀₋₂₄ (µg/ml/h), AUC_{0-∞} (µg/ml/h) AUMC₀₋₂₄ (µg/ml/h), C_{max} (µg/ml), C_p⁰ (µg/ml), T_{max} (h), MRT (h) Oral bioavailability (F)%, Vd (L/kg), Biological t_{1/2}(h), and Total body clearance (L/kg/h) by the LCMS/MS analytical technique through the non compartmental analysis PK solver soft ware system. In the present study, there was an increase in the values of AUC, AUMC, C_{max}, C_p⁰, t_{1/2} and MRT pharmacokinetic parameters after oral administration compared to the previous studies in the dual purpose chicken.

Keywords: Dual purpose chicken, levofloxacin, oral route, pharmacokinetic

Introduction

Fluoroquinolones are important group of antimicrobial drugs used in veterinary medicine. They have broad-spectrum activity against bacteria, mycoplasma and rickettsia (Brown, 1996) [1]. Fluoroquinolones are very potent antimicrobials and effective against a wide range of pathogenic organisms and are well distributed in the body after administration. They exhibit excellent oral bioavailability, extensive tissue penetration, low protein binding and long elimination half life (Brunton *et al.*, 2005) [2].

The fluoroquinolones act by inhibiting the DNA gyrase, thus interfering with the DNA-synthesis and inhibition of resealing leading to liberation of fragments that are subsequently destroyed by bacterial exonucleases (Sharma *et al.*, 2009) [3]. Fluoroquinolones were approved for 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.

Levofloxacin is a third generation fluoroquinolone with broad spectrum nature and is a levo isomer of ofloxacin (Martinez *et al.*, 2006 [4]; Nelson *et al.*, 2007) [5]. Its spectrum of activity includes most strains of gram positive and gram negative anaerobic bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal tract, skin and soft tissue infections (Solomkin *et al.*, 2010) [6]. It has an excellent broad-spectrum activity against *Mycoplasma* and *Chlamydia* organisms in veterinary medicine (Aboubakr, 2012) [7].

Fluoroquinolones are collectively referred to as "respiratory quinolones", which exhibited modest activity towards important respiratory pathogen *Streptococcus pneumonia* (Wispelwey and Schafer, 2010) [8].

As compared to other fluoroquinolones, such as ofloxacin and ciprofloxacin, it also has more pronounced bactericidal activity against organisms such as *Pseudomonas*, *Enterobacteriaceae*

and *Klebsiella* (Klesel *et al.*, 1995) [9]. Levofloxacin is more extensively distributed into intrapulmonary compartments than ciprofloxacin and achieved significantly higher steady-state concentrations in plasma and epithelial lining fluid (Gotfried *et al.*, 2001) [10].

Levofloxacin along with other fluoroquinolones such as gatifloxacin, moxifloxacin, grepafloxacin, trovafloxacin offer more favourable pharmacokinetic parameters such as higher AUC, C_{max} and longer elimination half-life than older compounds such as ciprofloxacin. Levofloxacin is metabolized in the liver to demethyl-levofloxacin and levofloxacin-N-oxide and excreted through the urine (Lubasch *et al.*, 2000) [11]. The drug distributes well to the target body tissues and fluids in respiratory tract, skin, urine and prostate, and its uptake by cells makes it suitable for use against intracellular pathogens (Langtry and Lamb, 1998) [12].

The good bioavailability, large volume of distribution, high C_{max} , AUC and pharmacokinetic-pharmacodynamic hybrid efficacy predictors, adverse effects indicate that administration of levofloxacin at 10 mg/kg bw by different routes may be highly efficacious against susceptible bacteria in turkeys (Aboubakr *et al.*, 2014) [13].

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 (Gouvea *et al.*, 2015) [14].

Various analytical techniques such as atomic absorption, spectrometry, polarography, differential pulse polarography, capillary zone electrophoresis, spectrofluorometry and High Performance Liquid Chromatography (HPLC), liquid-liquid extraction, Solid phase extraction by Liquid Chromatography Mass Spectroscopy/ Mass Spectroscopy (LC- MS/MS) and Nuclear Magnetic Resonance (NMR) were used to extract metabolites and determination of antibiotic residue concentration in the animal tissues (Amjad *et al.*, 2005) [15].

There were few studies in the assessment of residual status of levofloxacin in the tissues of broiler chicken. There is no MRL level and withdrawal period fixed for the levofloxacin by regulatory agencies for birds. It is essential to generate tissue depletion data in order to arrive at conclusion regarding maximum residual limits (MRLs) and withdrawal period for levofloxacin drug. In view of the marked species variation in the pharmacokinetic data of antimicrobial drugs, present study was planned to determine the disposition kinetics, residue level and withdrawal period of levofloxacin following single oral at the dose of 10 mg/kg bw in Indian Rock-3, a strain of white plymouth rock dual purpose chicken. Keeping the above points in view, the present study was undertaken in dual purpose chicken (Indian Rock-3) with the following objectives, To study the pharmacokinetics of levofloxacin after single oral and intravenous dose administration in dual purpose chicken.

Materials and Methods

The pharmacokinetic parameters of levofloxacin were studied after oral administration was carried out in dual purpose chicken.

Experimental animals

The study was conducted in 30 to 35 day old (n= 10) healthy

dual purpose chicken Indian Rock-3 (IR-3), a strain of White Plymouth Rock developed by Karnataka Veterinary Animal and Fisheries Sciences University, Bidar (Fig.1). The study was performed at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru. The birds 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 °C) and at 45 to 65 percent relative humidity. Food and water were supplied *ad libitum* and standard managerial practices were followed to keep the birds free from stress. The prior approval of the Institutional animal Ethics Committee (IAEC) was obtained before the commencement of the experiment (LPM/IAEC/181/2014, Date: 10/01/2014).

Drugs and Chemicals

Levofloxacin hemihydrate Injection and oral solution 10% (Meriflox[®], Vetoquinol India Animal Health Private Limited, Mumbai, India) were used for the pharmacokinetic study. The Levofloxacin and Indomethacin technical grade powder were obtained from Vetoquinol, India Animal Health Private Limited, Mumbai and Sigma Aldrich, (Poole, UK) respectively were used for the standardization and calibration of the LC-MS/MS equipment for pharmacokinetic and residue analysis study (Fig.2).

Formic acid, acetic acid, methanol and acetonitrile (HPLC grade) were obtained 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, India).

Pharmacokinetics of levofloxacin in dual purpose chicken

The experimental birds (n=10) were randomly allocated to received single oral dose of the levofloxacin drug. The drug was administered at a dose rate of 10 mg/ kg bw through oral route directly in to the crop using a thin plastic tube attached to a syringe (Fig.3). Blood samples (1 ml) were collected using i.v catheter (Venflon, 22G x 25mm) fixed into wing vein and transferred to clean sterilized heparinized test tubes. Following oral administration, blood samples were collected at time 0 (before drug administration), 5, 10, 15, 30 and 45 min 1, 2, 3, 4, 6, 8, 12, 16 and 24 h. Plasma samples were separated soon after blood collection by centrifugation (3500 rpm for 10 min at 4 °C), stored at -20 °C until analysis of pharmacokinetic parameters. The blank plasma sample was used for the preparation of calibration and standardization of the LC-MS/MS equipment.

Estimation of pharmacokinetic parameters

Pharmacokinetic parameters like peak plasma concentration (C_{max}), C_p^0 (Time to reach peak concentration at zero hour), T_{max} , Area under the curve: AUC_{0-24} , $AUC_{0-\infty}$, $AUMC_{0-24/0-\infty}$, mean residence time (MRT), volume of distribution (Vd), biological half life ($t_{1/2}$) and total body clearance (Cl_B) were estimated using LC-MS/MS analytical equipment (Fig.2) and calculated the mean plasma concentration by linear trapezoidal with linear interpolation technique using PK Solver non compartmental analysis software program (Albarellos *et al.*, 2005) [16].

LC- MS/MS ANALYSIS

Principle

Chromatography is the ability to separate molecules using partitioning characteristics of molecule to remain in a stationary phase versus a mobile phase. High performance liquid chromatography (HPLC) is about solvent being forced through under high pressures of up to 400 atmospheres. That makes it much faster and allows very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

Liquid chromatography separation is influenced by the liquid solvent condition (1000-6000 psi), chemical interactions between sample mixture and liquid solvent (hydrophobicity, protonation), solid particles packed inside of the separation column (ligand affinity, ion exchange). The mass spectrometry used to separate gas phase ions according to their m/z (mass to charge ratio) value. The analyser uses electrical or magnetic fields, or combination of both, to move the ions from the region where they are produced, to a detector, where they produce a signal which is amplified. The analyser is operated under high vacuum, so that the ions can travel to the detector with a sufficient yield. The mass spectrometer, ionize the chemical compound through the Electrospray Ionisation (ESI), Atmospheric Pressure Chemical Ionisation (APCI) and Atmospheric Pressure Photoionization (APP) and generation of the charged molecule, measuring the charge to mass ratios and detect masses of all the chemicals present in the peak, which can be a very good starting point for identifying them, and an excellent method to check for purity of the compound.

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 components. Compounds that are thermally labile, exhibit high polarity or have a high molecular mass may all be analysed using LC-MS. The compounds were separated on the basis of their relative interaction with the chemical coating of these particles (stationary phase) and the solvent eluting through the column (mobile phase) and introduced to the mass spectrometer via a specialised interface to find the accurate mass of the chemical. It gives the clear idea about the presence of the chemical in starting mixture.

Experimental Conditions of LC-MS/MS

The chromatography was carried out with LC-MS/MS (Agilent Technologies, Waldbron, Germany) Agilent 1200 RRLLC 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. The separation was achieved using a gradient elution with the flow rate of 0.7 ml/min, while the injection volume was 20 µl.

Solvents used for LC-MS/MS analysis

Time (min)	A%	B%	Flow Rate (ml/min)
0.01	80	20	0.7
3.00	70	30	0.7

A: Acetonitrile, B: 0.1% v/v formic acid in water

The source/gas conditions were as under the curtain gas (CUR) was set at 40psi, while the ion source gas 1 (GS1) and ion source gas 2 (GS2) were set at 40 psi. The temperature was set at 20 °C. The conditions for the compound were Declustering Potential (50.0), Entrance Potential (10.0), Collision energy (30.0) and Collision cell exit potential (5.0). The mass spectrometer was operated in a multiple reaction monitoring (MRM) mode that selected one precursor ion and two product ions for each target compound.

Stock solutions

The main stock solutions of levofloxacin and indomethacin were prepared by dissolving the appropriate amount of each compound in methanol. The spiking stock solutions of levofloxacin and working stock solution of indomethacin were prepared by using diluent (Methanol: water, 50:50% v/v). All the stock solutions were stored at 2-8 °C.

Preparation of calibration standard solutions and quality control stocks

The primary stock solution of levofloxacin 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, 4000, 10000, 20000, 40000, 80000, 120000, 160000 and 200000 ng/ml. These solutions were used to prepare relevant calibration curve (CC) standards. Another set of working solutions of levofloxacin was prepared in the diluent (from primary stock) at concentrations of 2000, 6000, 100000 and 180000 ng/ml respectively for QC samples (LLOQC, LQC, MQC and HQC). The calibration standards and quality control samples were prepared by spiking 0.01 ml of the spiking stock solution (levofloxacin) into 0.190 ml of screened blank chicken plasma. The calibration samples were made at concentrations of 100, 200, 500, 1000, 2000, 4000, 6000, 8000 and 10000 ng/ml. Quality control samples were prepared at concentrations of 100 ng/ml (Lower limit of quality control, LLOQC), 600.00 ng/ml (lower quality control, LQC) 5000 ng/ml (Medium quality control, MQC) and 9000.00 ng/ml (Higher quality control, HQC). di-Potassium Ethylene Diamine Tetra Acetic acid (K₂EDTA) anti coagulated whole chicken blood was centrifuged at 3500 rpm for 10min at 4 °C to separate plasma from erythrocytes. The plasma fraction was stored at -20±5 °C until pharmacokinetic analysis (Fig.3).

Chromatographic conditions

The mobile phase was optimized through several trials to obtain good resolution. The presence of small amount of formic acid in the mobile phase improved the detection of analyte. It was found that acetonitrile (0.1% v/v): formic acid in water (80:20% v/v) could achieve this purpose and adopted as a final mobile phase. Agilent Column -8 RP, 4.6 mm*50, 5 µm column resulted in providing good peak shapes and response at lowest concentration level. The mobile phase was operated at a flow rate 0.4 ml/min. The retention time for

levofloxacin and indomethacin was 0.96 and 1.72 min respectively. The chromatographic run time was 2.4 min. The indomethacin was used as an internal standard, because the chemical formula, structure, physicochemical properties like pH, pka and molecular mass were similar to the of levofloxacin drug.

Selectivity and chromatography

The degree of interference by endogenous plasma constituents with the analyte and internal standard was assessed by the inspection of chromatograms derived from the processed blank plasma sample. The respective chromatograms of blank sample, extracted lower limit of quantification and upper limit of quantification samples. There was no interference observed in the blank plasma sample at the retention time of the analyte and internal standard (Fig 4).

Sensitivity

The lowest limit of reliable quantification for analyte was set at the concentration of the LLOQ. The precision and accuracy at LLOQ concentration was 15.65% and 102.33% respectively.

Linearity

The nine point calibration curve was found to be linear over the concentration range of 100 -10000 ng/ml. After weighing factor of $1/x$ and $1/x^2$, a regression equation with a weighing factor of $1/x^2$ of drug to internal standard concentration was found to produce the best fit concentration response relationship for the analyte in chicken plasma. The mean correlation coefficient of the weighted calibration curves generated during the validation was 0.99 (Fig. 10 and Table 1).

Ion mass spectra of levofloxacin

The mass parameters were tuned in both positive and negative ionization modes for the analyte and internal standard. Good response was found in positive ionization mode. The most sensitive mass transition monitored were 363.20 m/z to 319.30 m/z and 357.70 m/z to 139.10 m/z for levofloxacin and indomethacin respectively (Fig. 4).

Method validation

The method was validated for specificity/selectivity, linearity, precision and accuracy, recovery and stability as per United State Food and Drug Administration guidelines.

Specificity

For the study of specificity, which is the ability to differentiate between target analytes and interference, was assessed by analyzing three blank tissue samples. The analytes were identified by matching retention times of peaks with the values of the corresponding standard analyzed under the same experimental conditions.

Selectivity

The selectivity was determined by analyzing three replicates of blank tissue samples spiked with the lowest level of the calibration curve concentration.

Linearity

The linearity was tested for levofloxacin in the concentration range of 1-100 ng/ml. Standard calibration curves containing

at least nine points (non zero standards) were plotted and analyzed in triplicate for the determination of linearity. The blank tissue samples were also analyzed to confirm the absence of direct interference. The acceptance limit of accuracy for each of the calculated concentration was $\pm 15\%$ except for LLOQ where it was $\pm 20\%$. For a calibration run to be accepted at least 75% of the calibration standards, including ULOQ and LLOQ were required to meet the acceptance criterion.

Precision and accuracy

Inter-day assay

Inter-day assay precision and accuracy was determined by analyzing six replicates at four different Quality Control (QC) levels on two different day batches. The acceptance limit of accuracy was $\pm 15\%$ except for LLOQ where it was $\pm 20\%$ and precision of $\pm 15\%$ coefficient of variance (% CV) except for LLOQ, where it was $\pm 20\%$.

Intra-day assay

Intra-day assay precision and accuracy was determined by analyzing six replicates at four different QC levels on same day batches. The acceptance limit of accuracy was $\pm 15\%$ except for LLOQ where it was $\pm 20\%$ and precision of $\pm 15\%$ coefficient of variance (% CV) except for LLOQ where it was $\pm 20\%$.

Recovery

The recovery of the levofloxacin from the extraction procedure was determined by comparing the peak area of the analytes in spiked tissue samples (extracted samples) (three each of low, medium and high quality controls) with those of the analytes in tissue samples prepared by spiking the extracted analyte-free tissue samples with the same amounts of the analytes at the step immediately prior to chromatography (post spiked samples). Similarly recovery of the internal standard was determined by comparing the mean peak areas of the extracted QC samples with that of post spiked quality control samples at the step immediately prior to chromatography. The recovery of the analytes and internal standard should be at least more than 50% and reproducible response (Fig.6).

Stability test

The stability test was determined at room temperature and refrigerated conditions (aqueous at 2-8 °C and plasma samples at -20 °C). The acceptance coefficient of variance (% CV) limit for accuracy was $\pm 15\%$ and precision of $\pm 15\%$ for LQC and HQC samples.

Preparation of plasma samples

The plasma (200 μ l) was spiked with 10 μ l of Internal standard (Indomethacin) (40 μ g/ml), 200 μ l of 1% formic acid. The mixture was vortex-mixed for three min. The extraction was done by solid phase extraction (SPE) which involves four steps i.e conditioning, loading, washing and eluting. The cartridges were fixed to solid phase extraction set up and the cartridges were conditioned with 1ml of methanol, again the cartridges are conditioned with 1ml of water for two times. The spiked plasma samples were loaded and applied negative pressure. Washing of cartridges are done with two washings, first wash with 1ml of water and second wash with 1 ml of 5% methanol. The analyte is eluted into) Radio

Immuno Assay (RIA) vails by adding 200 µl of mobile phase (acetonitrile: 0.1% formic acid). An aliquot of 20 µl was injected into the LC-MS/MS system for analysis. All the data were calculated by Pharmacokinetic (PK) Solver soft ware.

Chromatograms of levofloxacin in pharmacokinetic study

After the stability of the LC-MS/MS equipment, the chromatogram of pharmacokinetic parameters at the different time intervals for both oral administration of the levofloxacin were depicted (Fig.7 and 8).

Results

Pharmacokinetic study through oral and intravenous route administration, residual analysis, withdrawal period and safety evaluation of levofloxacin following repeated oral administration in dual purpose chicken are shown here.

Pharmacokinetic study

The Liquid Chromatography- Mass Spectrometry (LC-MS/MS) method was used to measure plasma concentration time profile of levofloxacin in dual purpose chicken after oral administration at the dose of 10 mg/kg. The plasma samples were analyzed up to 24 hours for pharmacokinetic analysis. All the data were calculated by non compartmental model PK solver software.

Pharmacokinetics of levofloxacin following oral dose administration at 10 mg/kg bw in dual purpose chicken

The plasma concentration – time profile of levofloxacin following single oral dose in dual purpose chicken were depicted in Table .1 and Fig .9.

The mean plasma concentration (µg/ml) of levofloxacin were 0.00, 2.84±0.63, 3.78±0.16, 5.39±0.29, 5.81±0.35, 6.27±0.66, 6.81±0.53, 3.03±0.06, 2.32±0.12, 1.08±0.09, 0.63±0.14, 0.38±0.10, 0.17±0.05 and 0.06±0.01 µg/ml at 0 min, 5 min, 10 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 16 h and 24 h respectively.

The mean peak plasma concentration was 6.81±0.53 µg/ml attained at 1.0 h after the administration of levofloxacin. The pharmacokinetic parameters describing the disposition kinetics of levofloxacin following single oral dose administration were presented in (Table 1 and Fig 5).

The plasma concentration (mean±SE) values of levofloxacin in the present study were AUC₀₋₂₄:25.31±0.16 µg/ml/h, AUC_{0-∞}: 25.76±0.24 µg/ml/h, AUMC₀₋₂₄: 117.75±1.24 µg/ml/h, C_{max}: 6.81±0.53 µg/ml, T_{max}: 1.00±0.38 h, MRT :6.84 h±0.64 h, Oral bioavailability (F) : 84.4±1.62%, V_{dss}: 2.67±0.76 L/kg, Half life (t_{1/2}): 4.77±0.24 hand Total body clearance : 0.03±0.02 L/kg/h (Table. 2)

Discussion

The study was carried out to evaluate the pharmacokinetic parameters, residual level and the withdrawal period of levofloxacin in dual purpose chicken after single oral and i.v dose administration.

Pharmacokinetics of levofloxacin following oral administration (10 mg/kg bw) in dual purpose chicken

Area under curve

In the present study, mean value of AUC₀₋₂₄ was 25.31±0.16 µg/ml/h. This finding is in accordance with Dimitrova *et al.* (2006) [17] who reported that AUC₍₀₋₂₄₎ value was 16.13±3.26 µg/h/ml after administration of enrofloxacin at 10 mg/kg bw

in broiler chicken.

Albarellós *et al.* (2005) [16] reported AUC₀₋₂₄ value was 46.63±19.67 µg/h/ml following administration of levofloxacin at the dose of 10 mg/kg of bw through oral route in cats. Haritova *et al.* (2006) [19] reported that AUC₀₋₂₄ value of 9.21±3.19 µg/h/ml following the administration of marbofloxacin at 2 mg/kg bw through oral route in turkeys.

In the present study, mean value of AUC_{0-∞} was 30.53±0.80 µg/ml/h. This value is in accordance with finding of Banna *et al.* (2013) [18] who reported that AUC_{0-∞} value was 24.62±1.78 µg/ml/h after administration of levofloxacin at 10 mg/kg bw in broiler chicken.

Haritova *et al.* (2006) [19] reported AUC₀₋₂₄ value of 10.89±3.21 µg/h/ml after the administration of marbofloxacin at a dose of 2 mg/kg bw through oral route in turkeys. Varia *et al.* (2009) [20] reported AUC_{0-∞} value was 6.70±0.08 µg/h/ml following the administration of levofloxacin at the dose of 10 mg/kg bw in broiler chicken.

In the present study, mean value of AUMC is 117.75±1.24 µg/ml/h. This finding is in accordance with Varia *et al.* (2009) [20] who reported that AUMC was 41.73±1.15 µg/ml/h after administration of levofloxacin at the dose of 10 mg/kg bw through oral route in broiler chicken.

Aboubakr and Soliman (2014) [21] reported AUMC value of 37.46±2.61 µg/ml/h after oral administration of levofloxacin at a dose of 10 mg/kg bw in ducks.

Atta and Sharif, (1997) [22] reported AUMC value of 489.93±25.73 µg/ml/h following administration of ciprofloxacin at a dose of 5 mg/kg bw in broiler chicken.

Oral Bioavailability

In the present study, oral bioavailability of levofloxacin was 84%. This finding is in accordance with Aboubakr and Soliman (2014) [21] who reported that bioavailability 73.56% for levofloxacin after administration at the dose of 10 mg/kg bw in ducks.

Banna *et al.* (2013) [18] reported oral bioavailability of 107.47% following the administration of levofloxacin at a dose of 10 mg/kg bw in broiler chicken. Kalaisevi *et al.* (2006) [23] reported oral bioavailability of 110% after the administration of ofloxacin at the dose 10 mg/kg bw broiler chicken.

C_{max} and T_{max}

In the present study, mean values of C_{max} and T_{max} were 6.81±0.85 and 1.00±0.38. The present findings are supported by several similar studies, Banna *et al.* (2013) [18] who reported that C_{max} and T_{max} were 3.27±0.13 µg/ml and 1.32±0.09 h after administration of levofloxacin at 10 mg/kg bw in broiler chicken.

The C_{max} and T_{max} were 5.15±0.12 µg/ml and 2.00±0.00 h after administration of levofloxacin at 10 mg/kg bw in turkeys (Aboubakr *et al.*, 2014) [24].

Aboubakr *et al.* (2014) [24] reported that C_{max} and T_{max} were 3.63±0.12 µg/ml and 2.05±0.08 h after administration of levofloxacin at 10 mg/kg bw in ducks.

Mean residence time (MRT)

In the present study, mean MRT value was 6.84 h. The result is in agreement with the findings of Varia *et al.* (2009) [20] who reported MRT value of 6.12±0.13 h following oral administration of levofloxacin at 10 mg/kg bw in broiler chicken. Banna *et al.* (2013) [18] reported MRT was 6.59±0.44

h after administration of levofloxacin at 10 mg/kg b.w in broiler chicken. Aboubakr *et al.*, (2014)^[24] reported MRT was 6.30 ± 0.13 h after administration of levofloxacin at 10 mg/kg bw in turkeys.

Volume of Distribution at steady state ($V_{d_{ss}}$)

In the present study, mean $V_{d_{ss}}$ value was 2.67 ± 0.90 L/kg. This result is in accordance to the findings of Kalaiselvi *et al.* (2006)^[23] who reported that volume of distribution ($V_{d_{ss}}$) of 2.16 L/kg after administration of ofloxacin at 10 mg/kg bw in broiler chicken.

Banna *et al.* (2013)^[19] reported that $V_{d_{ss}}$ was 3.25 ± 0.06 L/kg after administration of levofloxacin at 10 mg/kg b.w in broiler chicken. Aboubakr *et al.* (2014)^[24] reported $V_{d_{ss}}$ was 1.31 ± 0.04 L/kg after administration of levofloxacin at 10 mg/kg bw in turkeys.

Elimination half life ($t_{1/2(\beta)}$)

In the present study, elimination half life ($t_{1/2(\beta)}$) was 4.77 ± 1.82 h. Similar studies have also shown that Aboubakr *et al.* (2014)^[24] reported the $t_{1/2(\beta)}$ was 4.07 ± 0.17 h after administration of levofloxacin at 10 mg/kg bw in turkeys. Kalaiselvi *et al.* (2006)^[23] reported that elimination half-life was 5.85 h following administration of ofloxacin at 10 mg/kg bw in broiler chicken. Aboubakr and Soliman (2014)^[21] reported that $t_{1/2(\beta)}$ was 3.94 ± 0.14 h after administration of levofloxacin at 10 mg/kg b.w in muscovy ducks. Varia *et al.* (2009)^[20] reported that $t_{1/2(\beta)}$ was 3.64 ± 0.15 h after administration of levofloxacin at 10 mg/kg bw in broiler chicken.

Total body clearance (Cl_B)

In the present study, total body clearance (Cl_B) was 0.03 ± 0.06 L/h/kg. The present finding is supported by Albarellos (2005)^[16] who reported that the total body clearance of 0.14 ± 0.04 L/h/kg following the administration of levofloxacin at 10 mg/kg bw in cats.

In the present study, there was an increase in values of pharmacokinetic parameters (AUC, AUMC, $V_{d_{ss}}$, $t_{1/2}$, C_{max} , C_p^0 and T_{max}) after oral administration compared to the earlier studies because estimation of pharmacokinetic parameters done by LC-MS/MS equipment, the methods being sensitive, specific and accurate compared to conventional analytical methods like HPLC and microbiological assay methods.

The levofloxacin was found to more rapidly absorbed, widely distributed and more quickly eliminated than other fluoroquinolones in the dual purpose chicken. The AUC value is directly proportional to the dose and inversely with the clearance but independent on the volume of distribution. The high value of the AUC reflects a vast area of the body is covered by drug concentration. The volume of distribution suggestive of good penetration of levofloxacin drug through the biological membranes and tissues. The extensive distribution of the drug into various body fluids and tissues due to higher V_d area. (Dumka and Srivastava, 2006)^[25]. The renal clearance of drug directly proportional to volume of distribution, rate of elimination and inversely proportional to the plasma drug concentration in birds (Aboubakr and Soliman, 2014)^[21].

In the present study, elimination half-life of levofloxacin in dual purpose chicken was slightly increased compared to the

earlier research findings. The levofloxacin is highly lipid soluble drug so slowly eliminated than other fluoroquinolones in broiler chickens. However elimination half life was lower than ciprofloxacin 9.01 ± 0.79 h (Atta and Sharif, 1997)^[22] because levofloxacin is rapidly eliminated than ciprofloxacin in broiler chickens. In conclusion good bioavailability, large volume of distribution, high C_{max} , C_p^0 , AUC and pharmacokinetic–pharmacodynamic hybrid efficacy predictors for levofloxacin indicated that administration of levofloxacin at 10 mg/kg and 8 mg/kg bw through oral route may be highly efficacious against susceptible bacteria in dual purpose chicken.



Fig 1: Strain of White Plymouth Rock



Fig 2: LC-MS/MS Equipment



Fig 3: Oral administration of Levofloxacin in birds

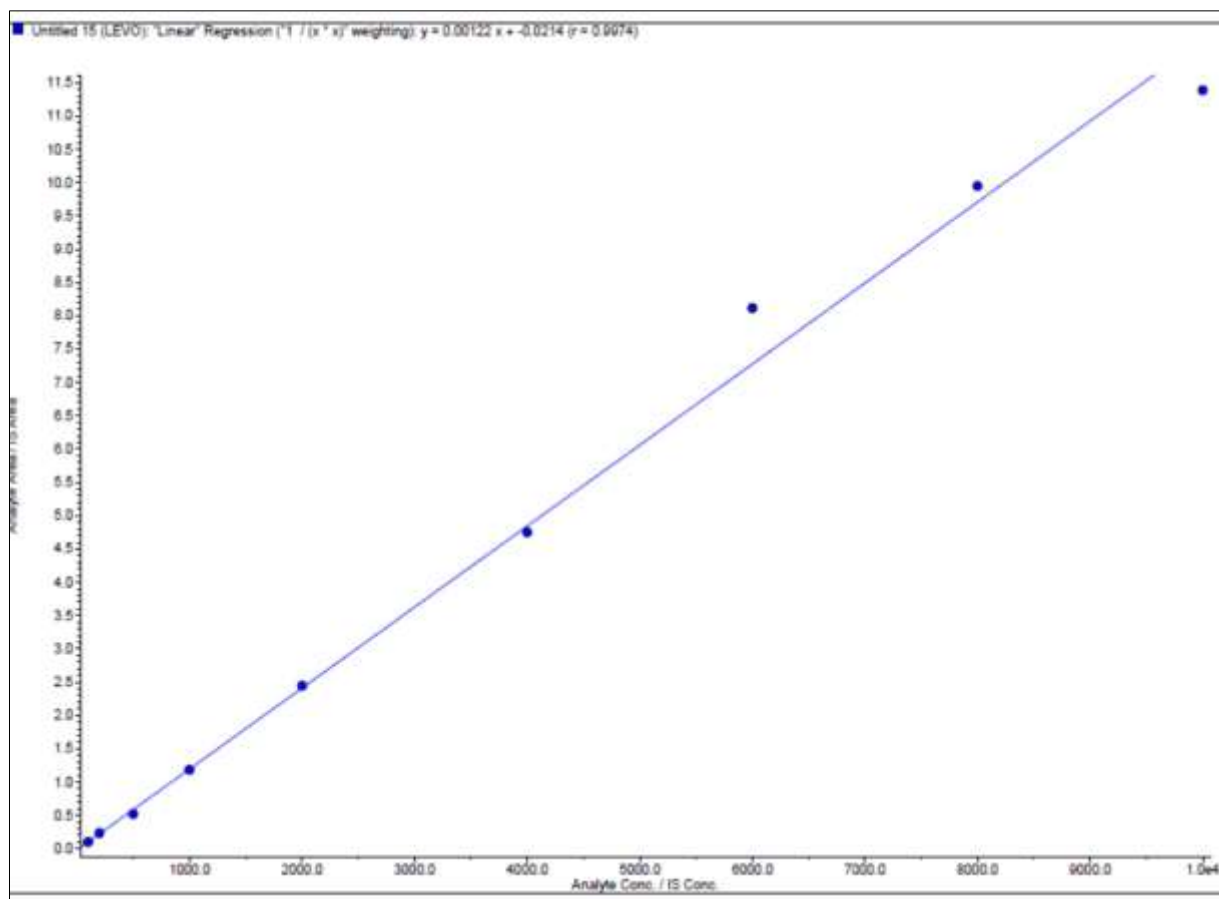


Fig 4: Linearity of the standard calibration curve of levofloxacin in chicken plasma (100 to 10000 ng/ml)

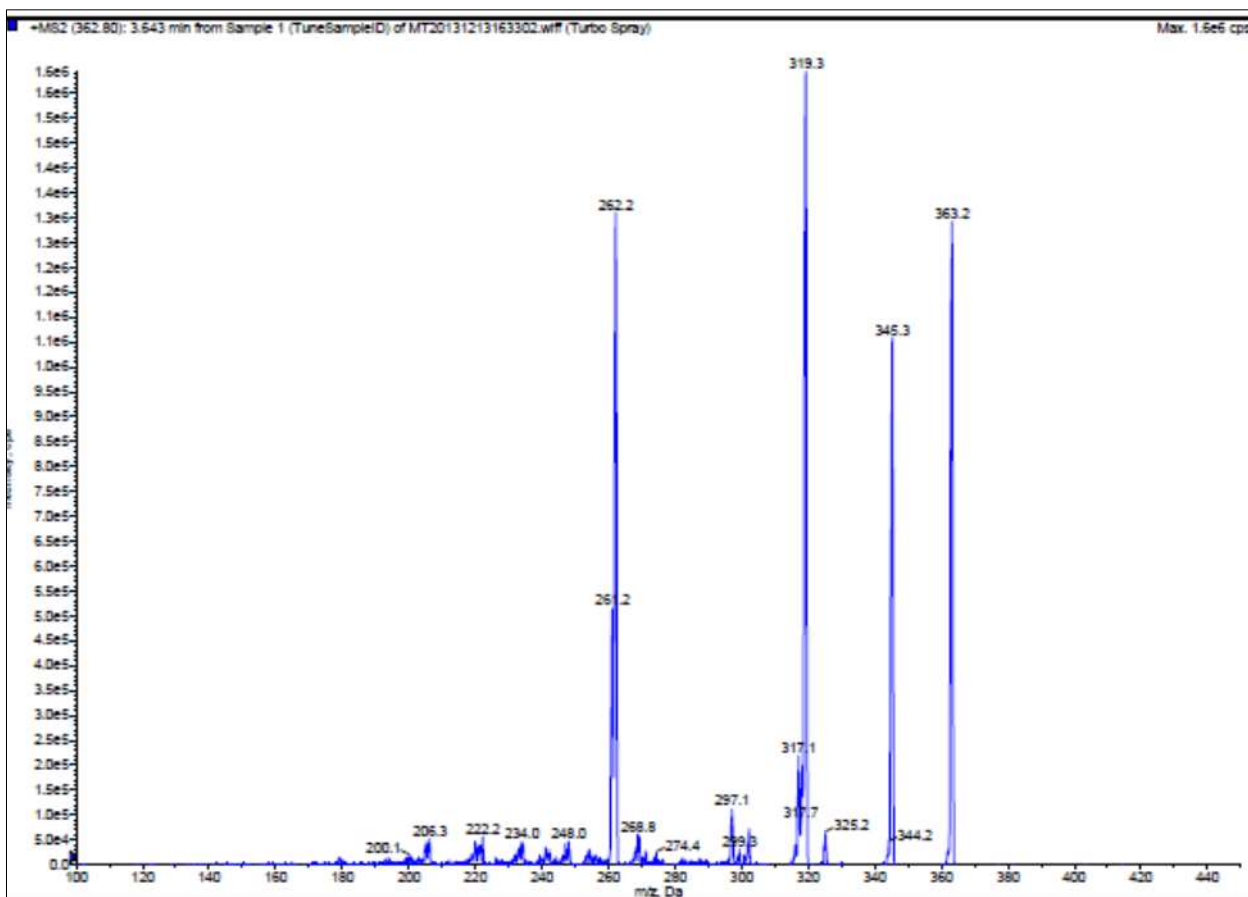


Fig 5: Product ion mass spectra of levofloxacin

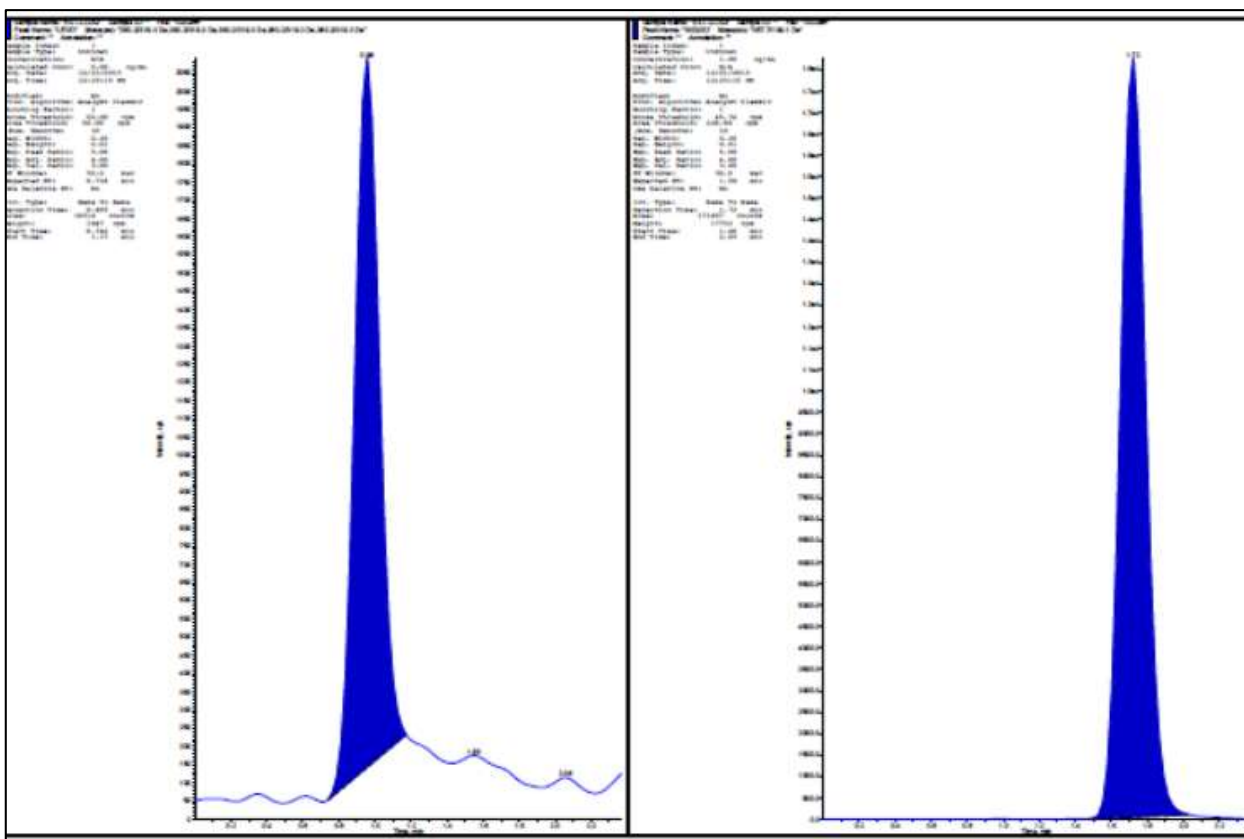


Fig. 6: Chromatogram of lower limit of quantification and internal standard (Indomethacin)

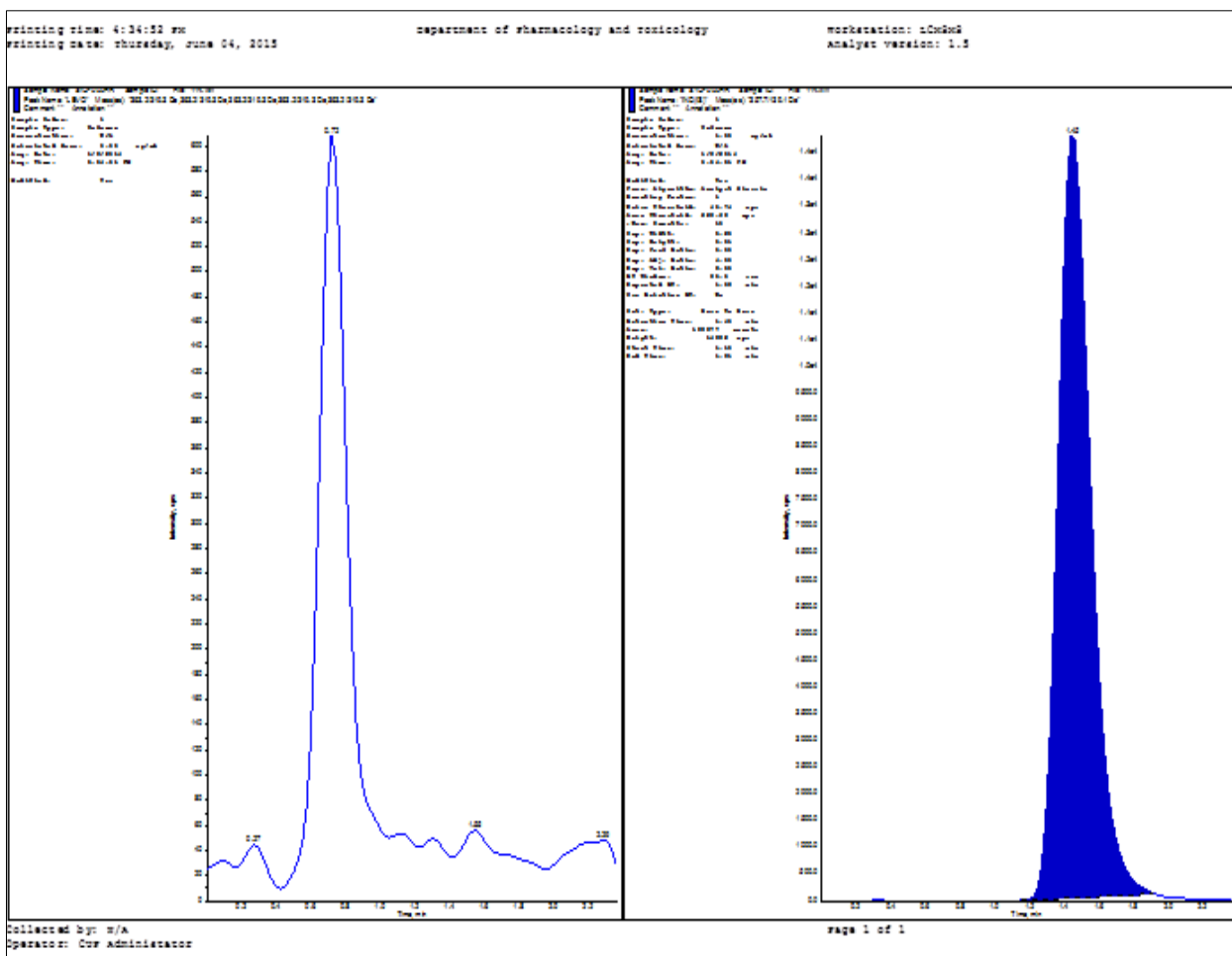


Fig 7: Chromatogram of levofloxacin after oral route administration (plasma: 5 min)

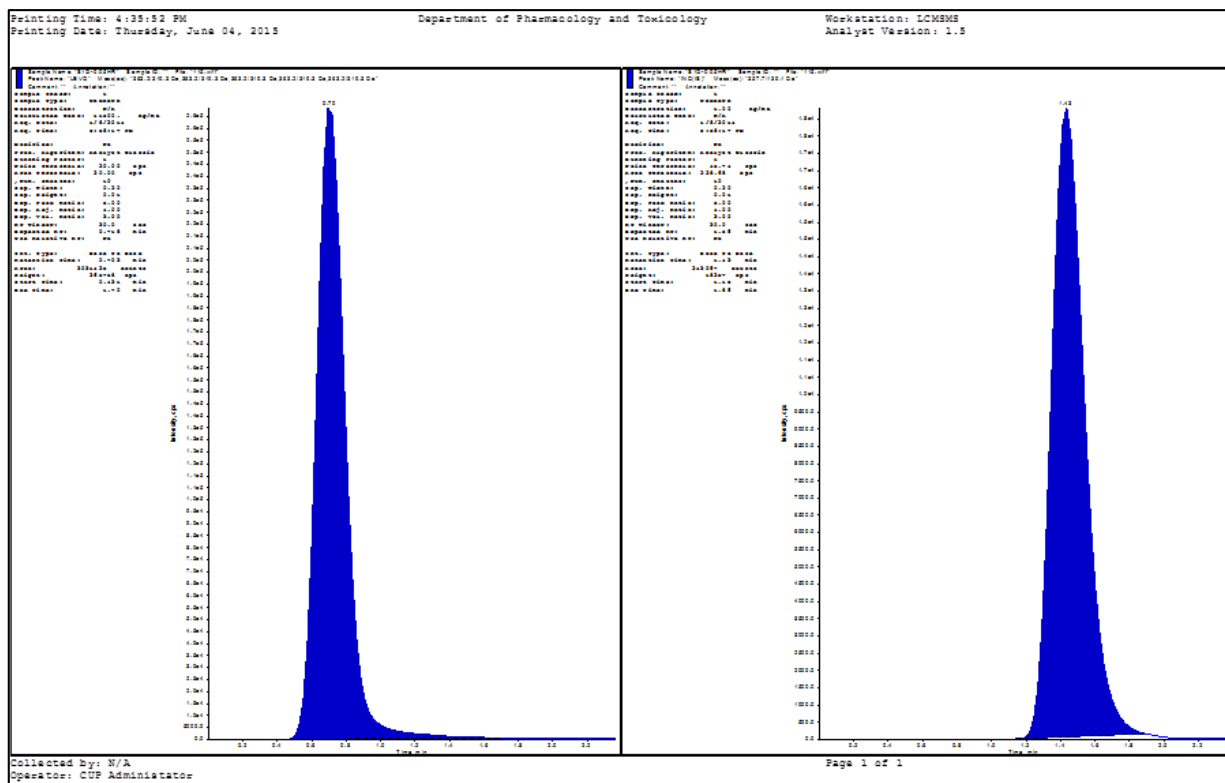


Fig 8: Chromatogram of levofloxacin after oral route administration (plasma: 10 min)

Table 1: Mean plasma concentration - time profile of levofloxacin at 10 mg/kg bw, oral route

Time	Concentration of levofloxacin ($\mu\text{g/ml}$) (Mean \pm SE)
0 min	0.00 \pm 0.00
5 min	2.84 \pm 0.28
10 min	3.78 \pm 0.16
15 min	5.39 \pm 0.29
30 min	5.81 \pm 0.35
45 min	6.27 \pm 0.66
1 h	6.81 \pm 0.53
2 h	3.03 \pm 0.06
4 h	2.32 \pm 0.12
6 h	1.08 \pm 0.09
8 h	0.63 \pm 0.14
12h	0.38 \pm 0.05
16h	0.17 \pm 0.04
24 h	0.06 \pm 0.01

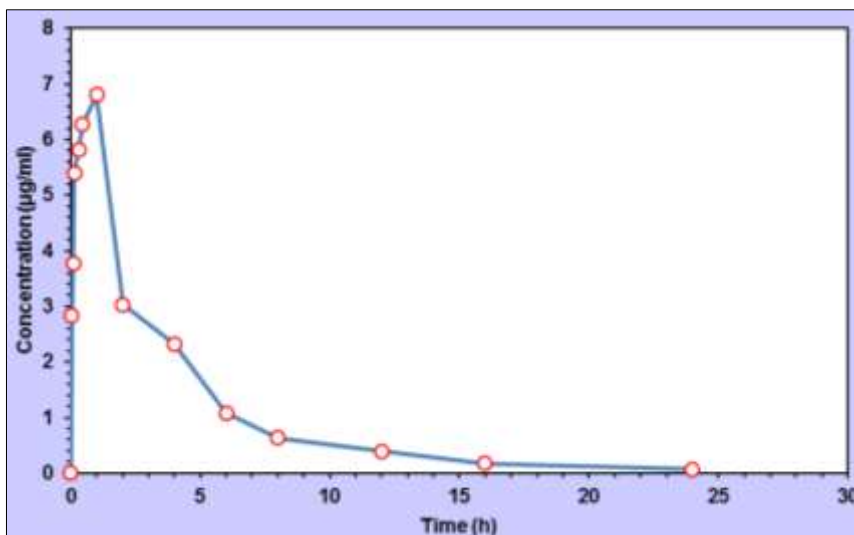


Fig 7: Plasma concentration– time profile of levofloxacin at 10 mg/kg bw, oral route

Table 2: Pharmacokinetic parameters of levofloxacin at 10 mg/kg bw oral route

Parameter	Unit	Value
$t_{1/2}$	h	4.77±0.24
T_{max}	h	1.00±0.38
C_{max}	µg/ml	6.81±0.85
AUC ₀₋₂₄	µg/ml/h	25.31±0.16
AUC _{0-∞}	µg/ml/h	25.76±0.24
AUC _{0-24/0-∞}		0.98±0.01
AUMC	µg/ml/h	117.75±1.24
MRT	h	6.84±0.64
Vd _{ss}	L/kg	2.67±0.76
Cl _B	L/h/kg	0.03±0.01
F	%	84.40±1.62

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