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The LCMS/MS analytical technique method for determination of pharmacokinetics of levofloxacin through intravenous route of administration in dual purpose chicken

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Abstract
The present study was conducted on Pharmacokinetics of Levofloxacin Through intravenous route of Administration in Dual Purpose Chicken by the LCMS/MS Analytical Technique. The experimental birds (n=10) were randomly allocated to receive single i.v dose of the levofloxacin drug. The levofloxacin drug was administered at a single dose rate 8 mg/kg bw through i.v administration, Following i.v 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. The samples were analysed by LC-MS/MS equipment using PK solver system. In the present study, there was an increase in the values of AUC, AUMC, C_{max}, C^0_p, $t_{1/2}$ and MRT pharmacokinetic parameters after i.v administration compared to the previous studies in the dual purpose chicken. In conclusion good bioavailability, large volume of distribution, high C_{max}, $C_p{}^o$, AUC and pharmacokinetic–pharmacodynamic hybrid efficacy predictors for levofloxacin indicated that administration of levofloxacin at 8 mg/kg bw through i.v route may be highly efficacious against susceptible bacteria in dual purpose chicken.

Keywords: Levofloxacin, intra venous route, dual purpose chicken

Introduction
Fluoroquinolones are collectively referred to as "respiratory quinolones", which exhibited modest activity towards important respiratory pathogen *Streptococcus pneumonia* (Wispelwey and Schafer, 2010) [1]. 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) [2]. It also has more pronounced bactericidal activity particularly against organisms such as *Pseudomonas*, *Enterobacteriaceae* and *Klebsiella* spp (Klesel *et al.* 1995) [3]. The bactericidal effect of levofloxacin is achieved through reversible binding to DNA gyrase and subsequent inhibition of bacterial DNA replication and transcription (Fu *et al.* 1992) [4]. The levofloxacin distributes well to target body tissues, fluids and its uptake makes it suitable for use against intracellular pathogens. However, it penetrates poorly in to central nervous system (Langtry and Lamb, 1998) [5]. It has an excellent broad-spectrum activity against *Mycoplasma* and *Chlamydia* organisms in veterinary medicine (Aboubakr, 2012) [6].

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) [7].

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) [8]. 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) [9].

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) [10]. Food-producing animals are treated with a variety of veterinary drugs, which can be administered in the feed or in the drinking water. The drugs areused in animal husbandry for different reasonsand may lead to residues in milk, eggs and other edible tissues (WHO, 1998) [11]. These residues may include non-altered parent compound as well as metabolites ortheir conjugates, have direct toxic effects on consumers or may cause problems indirectly through selection of resistant strains of bacteria (Fabrega *et al.,* 2009) [12].

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) [13].

In view of the marked species variation in the pharmacokinetic data of antimicrobial drugs, present study was planned to determine study the pharmacokinetics of levofloxacin after single intravenous dose administration in dual purpose chicken.

Materials and Methods

The pharmacokinetics of levofloxacin after the single dose intravenous 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. 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 managemental 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 (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 usedfor the standardization and calibration of the LC-MS/MS equipment for pharmacokinetic and residue analysis study.

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 were randomly allocated to receive either single i.v of the levofloxacin drug. The drug was administered at a dose rate of of 8 mg/kg bw single dose through a wing vein using a needle (22G x 25 mm). Birds were fasted for 12 hours before administration of the drug. Blood samples (1 ml) were collected using i.v catheter (Venflon, 22G x 25 mm) fixed into wing vein and transferred to clean sterilized heparinized test tubes.

Following i.v 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^0_p (Time to reach peak concentration at zero hour), T_{max}, Area under the curve: $AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$ $AUMC_{0\text{-}24/\ 0\text{-}\infty}$, 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 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) [14].

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 Electrospay Inosisation (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 specialized 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 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. 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, MQCand 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) (Fig. 1, 2, 3, 4 and 5). di- Potassium Ethylene Diamine Tetra Acetic acid (K_2EDTA) 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.

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 µ 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. 1)

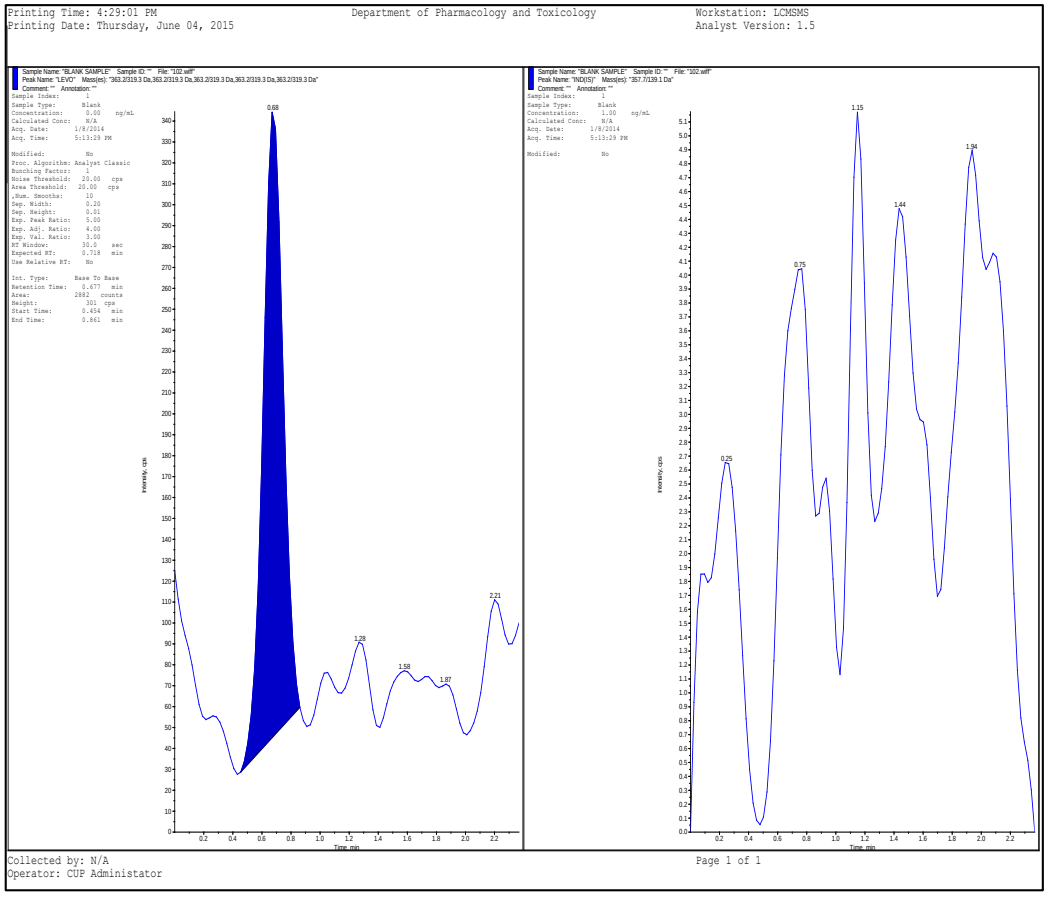

Fig 1: Chromatograh of blank sample and internal standard (Indomethacin)

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 2).

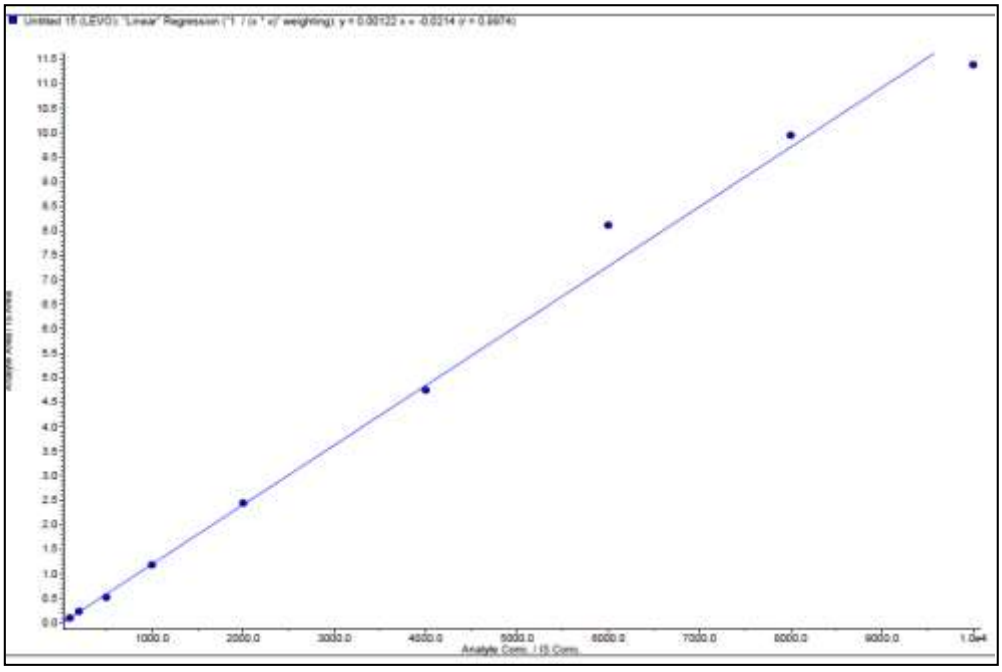

Fig 2: Linearity of the standard calibration curve of levofloxacinin chicken plasma (100 to 10000 ng/ml)

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.2).

Method validation

The method was validated for specificity/selectivity, linearity, precision and accuracy, recovery and stability as per United State Food and Drug Administration (USFDA, 2012) [15] 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±15% except for LLOQ where it was±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±15% except for LLOQ where it was±20% and precision of±15% coefficient of variance (% CV) except for LLOQ, where it was±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±15% except for LLOQ where it was±20% and precision of±15% coefficient of variance (% CV) except for LLOQ where it was±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.

Stability test

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

Preparation of plasma samples

The plasma (200 µl) was spiked with 10 µl of Internal standard (Indomethacin) (40 µg/ml), 200 µl 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 1ml of 5% methanol. The analyte is eluted into) Radio Immuno Assay (RIA) vails by adding 200µl 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.

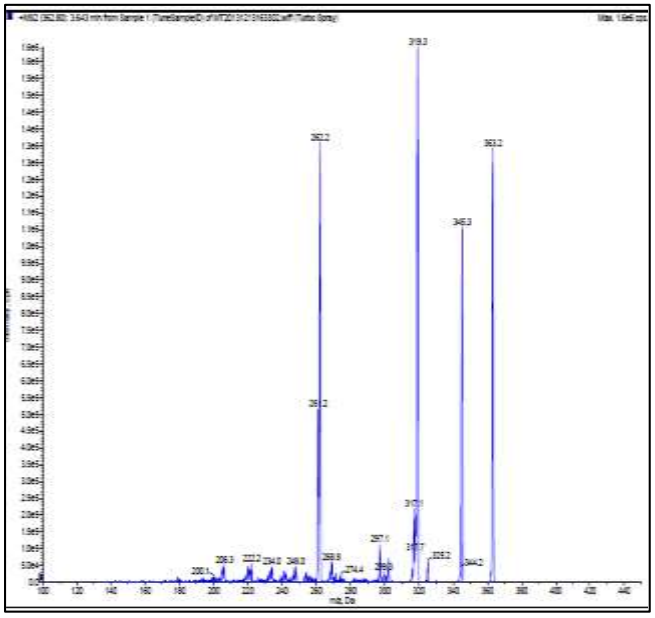

Fig 3: Product ion mass spectra of levofloxacin

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 i.v administration of the levofloxacin were depicted (Fig. 4 and 5).

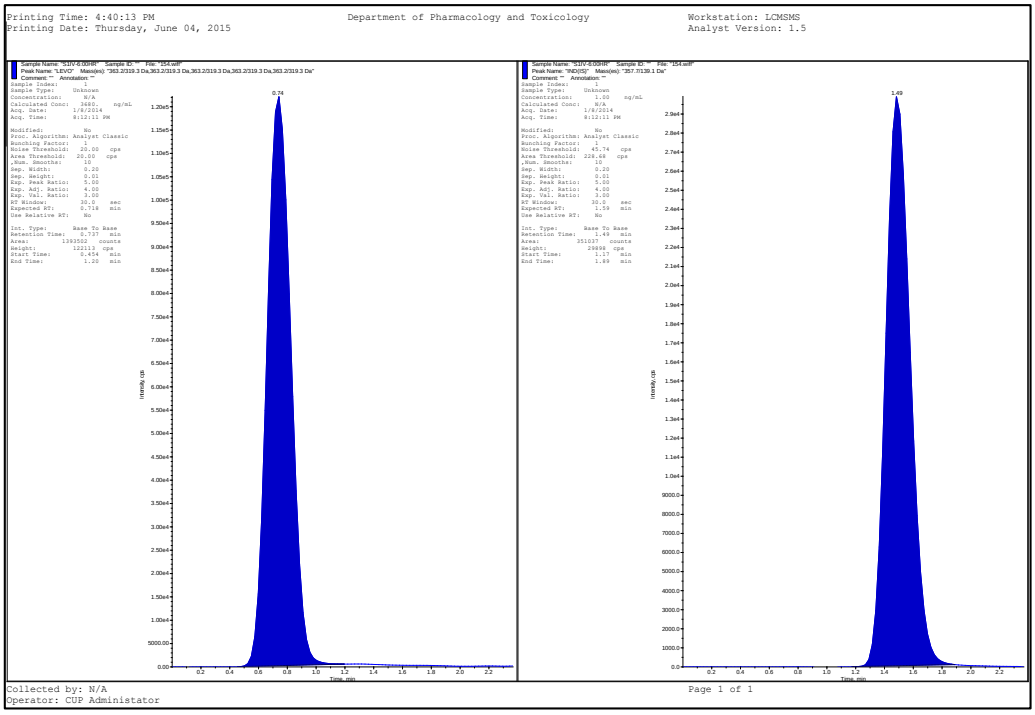

Fig 4: Chromatogram of levofloxacin after i.v administration (plasma: zero min)

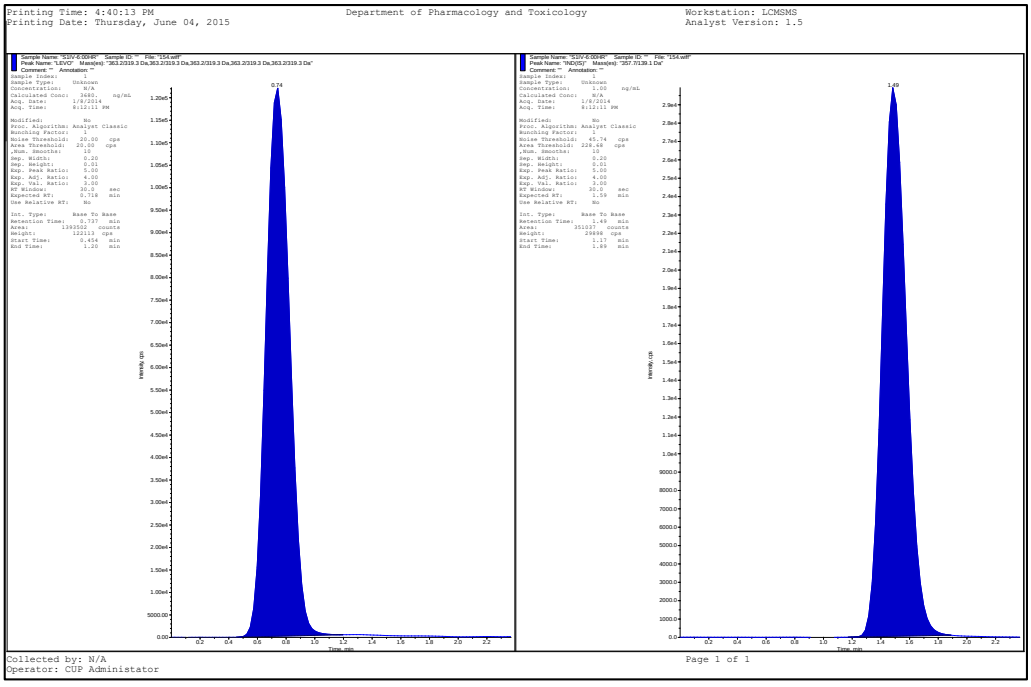

Fig 5: Chromatogram of levofloxacin after i.v administration (plasma: 12h)

Result

Pharmacokinetic study through intravenous route administration in dual purpose chicken is 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 i.v administration at the dose of 8 mg/kg bw. 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 i.v administration at 8 mg/kg bw in dual purpose chicken

The plasma concentration - time profile of levofloxacin following single i.v dose in dual purpose chicken were depicted in Table.

The mean plasma concentration (µg/ml) of levofloxacin were 8.45±0.40, 7.43±0.79, 6.80±0.42, 5.21±0.71, 4.30±0.47, 3.80±0.12, 3.26±0.26, 2.85±0.10, 2.29±0.08, 1.98±0.30, 1.24±0.11, 0.84±0.05, 0.62±0.02 and 0.08±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, 16h and 24 h respectively.

The mean peak plasma concentration was 8.45±0.40 µg/ml attained at zero hour after levofloxacin administration. The

pharmacokinetic parameters describing the disposition kinetics of levofloxacin following single i.v administration were presented in Table 1 and Fig. 6.

The plasma concentration (mean±SE) of levofloxacin values in the present study were AUC_{0-24}: 29.99±0.92 µg/ml/h,$AUC_{0-\infty}$:30.53±0.80 µg/ml/h, AUMC :208.90±1.08 µg/ml/h. The C^0_p :8.45±0.64 µg/ml, MRT:4.57±0.58 h, Vd_{ss}: 1.72±0.38 L/kg, Half life ($t_{1/2}$) : 4.56±0.96 h and Total body clearance:0.26±0.04 L/h/kg.

Table 1: Mean plasma concentration – time profile of levofloxacin at 8 mg/kg bw, i.v route

Time	Concentration of levofloxacin (µg/ml) (Mean±SE)
0 min	8.45±0.85
5 min	7.43±0.79
10 min	6.80±0.42
15 min	5.21±0.71
30 min	4.30±0.47
45 min	3.80±0.12
1 h	3.26±0.26
2 h	2.85±0.10
4 h	2.29±0.08
6 h	1.98±0.30
8 h	1.24±0.11
12h	0.84±0.05
16h	0.62±0.02
24 h	0.08±0.01

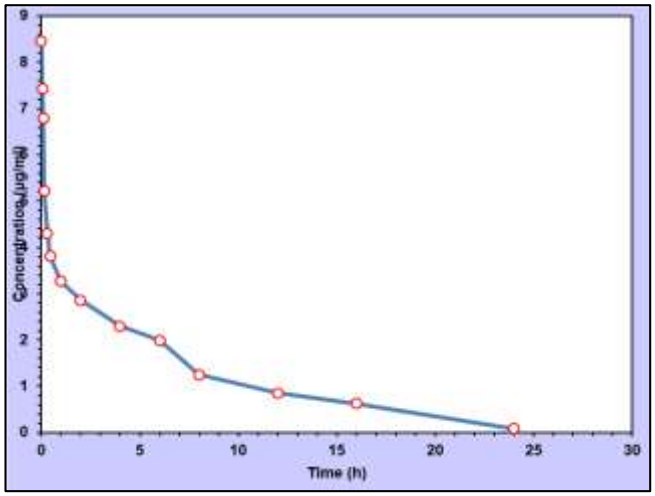

Fig 6: Mean plasma concentration – time profile of levofloxacin at 8 mg/kg bw, i.v route

Table 2: Pharmacokinetic parameters of levofloxacin at 8 mg/kg bw, i.v route

Parameter	Unit	Value
$t_{1/2}$	h	4.56±0.96
T_{max}	h	0.00±0.00
C_p^0	µg/ml	8.45±0.64
AUC 0-24	µg/ml/h	29.99±0.92
AUC 0-∞	µg/ml/h	30.53±0.80
AUC 0-24/0-∞		0.98±0.04
AUMC	µg/ml/h	208.90±1.08
MRT	h	4.57±0.58
Vd_{SS}	L/kg	1.72±0.38
Cl_B	L/h/kg	0.26±0.04

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 i.v dose administration.

Pharmacokinetics of levofloxacin following i.v administration (8 mg/kg b.w) in dual purpose chicken

Area under curve (AUC)

In the present study, mean AUC_{0-24} value was29.99±0.92µg/ml/h. The similar finding is reported by Goudah *et al*. (2008) [16] that $AUC_{0-24}$was 22.32±3.11 µg/ml/h after administration of levofloxacin at 4 mg/kg bw in lactating goat. Haritova *et al*. (2008) [17] reported that AUC_{0-24} was 11.79 µg/ml/h following administration danofloxacin at 24 mg/kg bw in turkeys.

In the present study, mean $AUC_{0-\infty}$was30.53±0.80 µg/ml/h. The similar findingis reported by Banna *et al*. (2013) [18] who reported that $AUC_{0-\infty}$ of 23.05±0.47 µg/ml/h after administration of levofloxacin at 10 mg/kg bw in broiler chicken. Arvind *et al*. (2013) [19] reported $AUC_{0-\infty}$ of 29.32±0.19 µg/ml/h after administration of levofloxacin at 10 mg/kg bw in cattle calves. Varia *et al*. (2009) [20] reported $AUC_{0-\infty}$ was 11.33±0.08 µg/ml/h after administration of levofloxacin at 10 mg/kg bw in broiler chicken.

In the present study, mean AUMC value was208.90±1.08 µg/ml/h. The similar finding is reported by Aboubakr *et al*. (2014) [21] who reported that AUMC was 225.43±34.56 µg/ml/h after administration of levofloxacin at 10 mg/kg bw in turkeys. Arvind *et al*. (2013) [19] reported the AUMC of 84.57±1.17 µg/ml/h after administration of 10 mg/kg bw in cattle calves. Varia *et al*. (2009) [20] reported the AUMC was 41.73±1.15 µg/ml/h after administration of levofloxacin at 10 mg/kg bw in broiler chicken.

Plasma drug concentration at zero hour (C^0_p)

In the present study, mean value was8.45±0.64 µg/ml, The present finding was supported by Banna *et al*. (2013) [18] who reported that C^0_p was 9.54±0.52 µg/ml after administration of levofloxacin at 10 mg/kg bw in broiler chicken. Aboubakr and Soliman (2014) [22] reported the C^0_p value of 13.93±0.44 µg/ml after administration of levofloxacin at 10 mg/kg bw in ducks. Aboubakr *et al*. (2014) reported C^0_p was 15.27±1.08 µg/ml after administration of levofloxacin at 10 mg/kg bw in turkeys.

Mean residence time (MRT)

In the present study, MRT was 4.57h. This finding was in accordance to the findings of Varia *et al*. (2009) [20] who reported that MRT of 3.69±0.08h after administration of levofloxacin at 10 mg/kg bw in broiler chicken. Banna *et al*. (2013) [18] reported MRT of 5.40±0.26 h after administration of levofloxacin at 10 mg/kg bw in broiler chicken. Aboubakr *et al*. (2014) [21] reported MRT was 5.20±0.30 h after administration of levofloxacin at 10 mg/kg bw in turkeys.

Volume of distribution at steady state (Vd_{ss})

In the present study, mean Vd_{ss} obtained was1.72±0.38 L/kg, The result is in agreement with the findings of Kalaiselvi *et al*. (2006) [23] who reported volume of distribution of ofloxacin as 1.76 L / kg after oral administration of levofloxacin at 10 mg / kg bwin broiler chickens. Banna *et al*. (2013) [18] reported $Vd_{ss}$was 2.36±0.13 L/ kg after administration of levofloxacin at 10 mg / kg bw in broiler chicken. Aboubakr *et al*. (2014) [21] reported that Vd_{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, mean elimination half life ($t_{1/2\beta}$) was 4.56±0.96 h. The result is in agreement with the findings of numerous studies, Kalaiselvi *et al.* (2006) [23] who reported that elimination half-life of ofloxacin was 4.46 h in broiler chickens administered with 10 mg/kg bw.

Banna *et al*. (2013) [18] reported $t_{1/2(\beta)}$value of 4.07±0.24 h after administration of levofloxacin at 10 mg/kg bw in broiler chicken. Varia *et al*. (2009) [20] reported that $t_{1/2(\beta)}$ value of 3.18±0.07 h after administration of levofloxacin at10 mg/kg bw in broiler chicken. Aboubakr and Soliman, (2014) [22] reported the $t_{1/2(\beta)}$ of 2.76±0.10 h after administration of levofloxacin at10 mg/kg bw in muscovy ducks.

Total body clearance (Cl_B)

In the present study, Total body clearance (Cl_B) was0.26±0.04 L/h/ kg. This finding is in accordance to the findings of Banna *et al*. (2005) [18] reported that total body clearance was 0.44±0.009 L/h/kg after administration of levofloxacin at 10 mg/kg bw in broiler chickens. Aboubakr and Soliman (2014) [22] reported that total body clearance was 0.41±0.04 L/h/kg after administration of levofloxacin at 10 mg/kg bw in muscovy ducks.

In the present study, there was an increase in values of pharmacokinetic parameters (AUC, AUMC, Vd_{ss}, $t_{1/2}$, C_{max}, C_p^o and T_{max}) after i.v 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 Vd area (Dumka and Srivastava, 2006) [24].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) [25].

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) because levofloxacin is rapidly eliminated than ciprofloxacin in broiler chickens.

Conclusion

In the present study, there was an increase in the values of AUC, AUMC, C_{max}, C^0_p, $t_{1/2}$ and MRT pharmacokinetic parameters after i.v administration compared to the previous studies in the dual purpose chicken. In conclusion good bioavailability, large volume of distribution, high C_{max}, C_p^o, AUC and pharmacokinetic–pharmacodynamic hybrid efficacy predictors for levofloxacin indicated that administration of levofloxacin at 8 mg/kg bw through i.v route may be highly efficacious against susceptible bacteria in dual purpose chicken.

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