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Pharmacokinetic profile of marbofloxacin following oral administration in broiler chickens

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Abstract
Marbofloxacin is a synthetic, third-generation fluoroquinolone drug, having concentration-dependent bactericidal activity and a broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria. Looking to the clinical potential of marbofloxacin to treat bacterial infections in poultry species, the present study was planned to investigate its pharmacokinetic profile following single dose oral administrations at a dose of 5 mg/kg body weight in eight broiler chickens. Plasma samples were analyzed for marbofloxacin concentration by Ultra High Performance Liquid Chromatography (UHPLC) with UV detector. The mean value of elimination rate constant (β) was 0.17 ± 0.03 h^{-1}. The mean value of its corresponding half-life ($t_{½\beta}$) was calculated to be 4.89 ± 0.65 h. The area under curve (AUC) of plasma marbofloxacin concentration *versus* time was found to be 14.70 ± 2.94 µg·h/ml. The mean values of mean residence time (MRT), apparent volume of distribution ($V_{d(area)}$) and total body clearance (Cl_B) were 7.48 ± 0.94 h, 2.80 ± 0.40 L/kg and 0.47 ± 0.11 L/h/kg, respectively. Marbofloxacin following single dose oral administration (5 mg/kg) showed favourable pharmacokinetic profile to be used clinically in broiler chickens.

Keywords: Broiler chickens, marbofloxacin, oral, pharmacokinetics, UHPLC

1. Introduction
A variety of antimicrobial agents were used in the past decades to treat different types of infections in poultry species. Therapeutic use of antibiotics has allowed poultry production to achieve significant improvement by mitigating the adverse effects of diseases and reducing mortality. Marbofloxacin is a third-generation fluorinated quinolone compound, which has a broad spectrum of antimicrobial activity against Gram-negative and Gram-positive bacteria [1]. It has also shown a significant post-antibiotic effect (PAE) and can kill bacteria during both stationary and growth phase of bacterial multiplication [2]. Marbofloxacin differs from other fluoroquinolones in chemical structure as the presence of an oxadiazine ring, which provides pharmacokinetic advantage such as a long elimination half-life [3]. It exhibits bactericidal action by inhibiting bacterial DNA topoisomerases II (gyrase) and IV, and by preventing the supercoiling of DNA [4]. Pharmacokinetic properties of marbofloxacin like the larger volume of distribution, optimum AUC and C_{max} make it a potentially useful drug for the treatment of genital tract, respiratory tract, gastrointestinal tract, skin and soft tissue infections in domestic animals and birds [5]. As with the other fluoroquinolones, marbofloxacin is a lipid-soluble organic acid with good tissue penetration. Because of its low MIC, broader spectrum of action and favourable pharmacokinetic profile, it may be extensively used in broiler chickens for the control and treatment of infectious diseases caused by susceptible microorganisms. Therefore, the present study was planned to investigate its pharmacokinetic profile following single dose oral administrations at a dose of 5 mg/kg body weight in eight broiler chickens.

2. Materials and Methods
2.1 Approval of project by ethics committee
This study was prior approved by Institutional Animal Ethics Committee (IAEC) of College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat vide proposal no. VETCOLL/IAEC/2022/19/PROTOCOL-13, in the month of January, 2022.

2.2 Experimental Animals
Eight healthy male broiler chickens (Vencobb strain) aged more than 2 weeks and weighing more than 1 kg were used in the present study. The broiler chickens for the present study were obtained from a registered commercial poultry farm, Banashkantha.

2.3 Experimental Location
Birds were kept at Laboratory Animal House, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar throughout the experimental period. Sardarkrushinagar (Dist: Banashkantha, Gujarat, India) is located at the latitude of 24°19‟34.3” North and a longitude of 72°19‟02.1” East. It is at an altitude of 154.52 meter above mean sea level and falls under a tropical and semi-arid climatic zone of North Gujarat, India. The study was conducted during March-April, 2022 when the ambient temperature ranged from 30 to 35 ℃.

2.4 Experimental Bird Management
Birds were housed in cages having water and feeding trough. Adequate feed and *ad libitum* water were provided to experimental birds throughout the experimental period (as per the standard schedule of CPCSEA guidelines for poultry/birds facility-2020). Birds were kept under constant observation for one week prior to the beginning of the experiment. All necessary management practices were followed so that the birds remained free from stress and diseases. In this period, they were subjected to daily clinical examinations to exclude the possibility of any disease.

2.5 Drugs and chemicals
Marbofloxacin powder of I.P. grade was procured from Nexia Enterprise, Mumbai, India. Water, methanol, formic acid, and acetonitrile of HPLC grade were purchased from S. D. Fine Chemicals Ltd., Mumbai. Perchloric acid (70-72%) was procured from the same manufacturer. All the chemicals were stored adequately and used prior to the expiry date.

2.6 Experimental design
Eight broiler chickens were administered marbofloxacin (5 mg/kg body weight, Oral route) through oral gavaging needle (16 G × 38 mm, curved needle). Periodical blood samples were collected from the wing veins of the birds in sterilized pre-heparinized small tubes at 0 (before drug administration) and 5 (0.083 h), 15 (0.25 h), 30 (0.5 h) min., 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 36 hours and 48 hours after administration of marbofloxacin. Approximately 0.5 ml of blood was withdrawn at each collection point. The plasma was separated after centrifugation of blood samples at 4000 revolutions per minute (RPM) for 10 minutes using a refrigerated centrifuge at 4 ℃. The plasma samples were transferred to cryo-vials (2 ml capacity) and then stored at -20 ℃ until assayed for marbofloxacin concentration using Ultra High Performance Liquid Chromatography (UHPLC) equipped with UV detector.

2.7 UHPLC analysis of marbofloxacin concentration
The plasma samples were analyzed for marbofloxacin concentration with minor modifications in a method as described by Carpenter *et al.* [7]. UHPLC apparatus (Thermo Fisher, Germany) consisting of UV detector (Dionex ultimate 3000), gradient solvent delivery pump (Dionex ultimate 3000) and manual injector was used for quantification of marbofloxacin from collected plasma samples. Chromatographic separation was performed by using reverse phase C_{18} analytical column (GL Science Inc., Japan, ODS-3V; 5 µm, 250 x 4.6 mm) at room temperature. The mobile phase consisted of mixture of 0.01 M formic acid (prepared in HPLC water) and acetonitrile (82:18), which was filtered by 0.45 µm size filter (Millipore®, Merck Life Science Pvt. Ltd., Bangalore) and degassed by ultra-sonication (Frontline Ultrasonic Cleaner, Ahmedabad, India). The mobile phase was pumped into column at a flow rate of 1.0 ml/min. Effluents were detected at 297 nm wavelength in UV detector.

2.8 Extraction of plasma samples
Deproteinization of each plasma sample (150 µl) was done by precipitation with addition of 150 µl of perchloric acid (20%). Mixture was vortexed for 1 minute followed by centrifugation at 10,000 rpm for 10 min at 4℃. Clear supernatant was collected in 2 ml eppendorf tubes. An aliquot of the resulting supernatant (50 µl) was injected in to UHPLC system through manual injector. Clear supernatant was collected in 2 ml eppendorf tubes. An aliquot of the resulting supernatant (50 µl) was injected in to UHPLC system through manual injector. The data integration was performed by “Chromeleon” software version 6.8. The marbofloxacin was detected from plasma at the average retention time of 5.6 minutes as depicted in Figure 1.

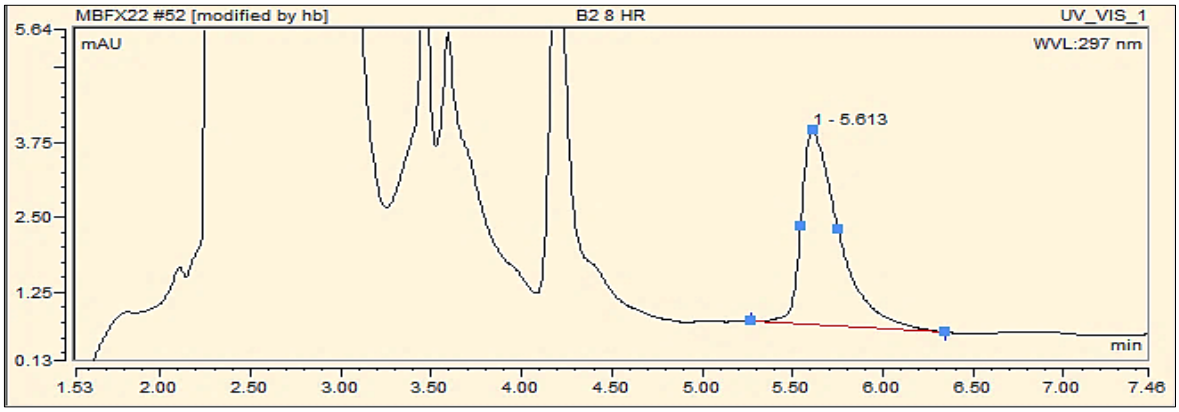

Fig 1: Chromatograms of plasma sample collected at 8 hours from Bird-2 (B2) after oral administration

2.9 Pharmacokinetic analysis
The plasma concentration-time curves of individual birds were subjected to non-compartmental analysis (NCA) for working out the pharmacokinetic parameters of marbofloxacin. Values of PK parameters were presented as a mean ± standard error (SE). The plasma concentration-time curves of individual broiler chickens were analyzed for obtaining PK parameters with the software “PK Solver 2.0”, a menu-driven add-in program for Microsoft Excel written in Visual Basic for Application (VBA) in solving basic problems in pharmacokinetic data analysis developed by Zhang *et al.* [15].

3. Results and Discussion

3.1 Plasma concentrations of marbofloxacin

The plasma concentrations of marbofloxacin at different time intervals following single dose oral administration @ 5 mg/kg body weight in broiler chickens (n=8) expressed as average values (mean ± SE) are presented in Table 1. The concentration *versus* time data series was plotted semi-logarithmically as depicted in Figure 2.

Table 1: Plasma concentrations of marbofloxacin following single dose oral administration (5 mg/kg b.wt.) in broiler chickens (n=8)

Collection time after drug administration (h)	Marbofloxacin concentration (µg/ml)								Mean ± SE
	Bird number								
	B1	B2	B3	B4	B5	B6	B7	B8	
0.083	0.42	0.10	0.35	0.67	0.22	0.14	0.22	0.30	0.30 ± 0.06
0.25	0.65	0.41	1.36	0.88	0.50	0.30	0.22	0.54	0.61 ± 0.13
0.5	1.21	1.06	1.54	1.24	1.05	0.91	0.97	0.74	1.09 ± 0.09
1	2.24	3.27	2.96	3.60	1.26	1.25	1.41	0.97	2.11 ± 0.37
2	1.87	1.32	2.63	3.08	1.25	0.55	1.25	0.94	1.61 ± 0.30
4	1.16	1.11	1.79	2.60	0.54	0.43	0.52	0.83	1.12 ± 0.26
8	0.73	0.72	1.48	1.08	0.19	0.29	0.43	0.45	0.67 ± 0.15
12	0.38	0.41	0.72	0.83	0.08	0.01	0.43	0.39	0.41 ± 0.10
24	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not Detected

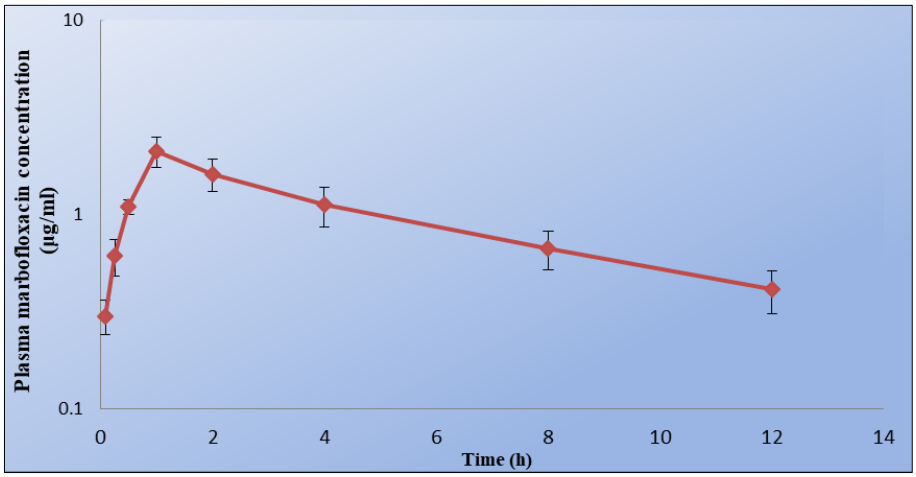

Fig 2: Semilogarithmic plot of mean ± SE concentration of marbofloxacin in plasma following oral administration (5.0 mg/kg body weight) in broiler chickens

Following oral administration of marbofloxacin, the drug concentration of individual birds at first time point of collection *i.e.*, at 0.083 h (5 minute) was observed to be ranging from 0.10 to 0.67 µg/ml with mean (± S.E.) value of 0.30 ± 0.06 µg/ml. The mean peak plasma drug concentration (C_{max}) of 2.11 ± 0.37 µg/ml was achieved at 1 h (T_{max}), which declined to 0.67 ± 0.15 µg/ml at 8 h and further to 0.41 ± 0.10 µg/ml at 12 h post drug administration. No drug concentration was detected in plasma samples collected post-12 h *i.e.* at 24, 36 and 48 h. The C_{max} values observed in the present study were higher than previously reported C_{max} of 1.05 ± 0.15 µg/ml after oral marbofloxacin administration in chickens [8]. Comparatively lower marbofloxacin C_{max} value of 1.37 µg/ml was also observed in chicken [9]. Relatively lower value of peak plasma drug concentration in horse @ 2 mg/kg b.wt. (0.89 µg/ml) was observed by Bousquet-Melou *et al.* [6]. It has been shown that C_{max} following oral administration in dog was increased from 0.831 ± 0.263 to 2.927 ± 0.581 µg/ml by increasing the dose from 1 to 4 mg/kg [11]. Higher peak plasma concentration after oral administration observed in present study can be attributed to higher dose of marbofloxacin (5 mg/kg).

3.2 Pharmacokinetic profile

Various PK parameters calculated from plasma concentration time profile after single dose oral administration of marbofloxacin (5 mg/kg body weight) in healthy broiler chickens are summarized in Table 2. In the present study, the elimination rate constant (β) was calculated as 0.17 ± 0.03 h^{-1} after single dose oral administration of marbofloxacin (5 mg/kg). After oral administration of marbofloxacin, a similar value of elimination rate constant (β: 0.16 h^{-1}) was reported in broiler chickens [11]. The mean value of elimination half-life ($t_{½β}$) after single dose oral administration of marbofloxacin (5 mg/kg) was found to be 4.89 h in broiler chickens in the present study. Similar $t_{½β}$ value in chickens (4.62 h) was observed [18]. The higher values of $t_{½β}$ were reported in broiler chickens (8.69 ± 1.17 h) by Anadon *et al.* [3], in turkey (6.23 ± 1.63 h) by Haritova *et al.* [10] and in quails (6.19 ± 0.08 h) by Aboubakr and Abdelazem [1]. The observed variation is common and as often as observed, because of species-specific elimination mechanisms, analysis techniques utilized, sampling time, well-being status and/or age of the biological entity [13].

The mean apparent volume of distribution ($V_{d(area)}$) following single dose oral administration of marbofloxacin (5 mg/kg) was calculated to be 2.80 L/kg in chickens. Patel *et al.* [12] reported lower values of $V_{d(area)}$ as, 1.49 L/kg in chickens. In contrast to this, lower $V_{d(area)}$ value of 0.96 ± 0.24 L/kg in cat and 1.81 ± 0.08 in goats were reported by Albarellos *et al.* [2] and by Bhardwaj *et al.* [5], respectively. The mean value of AUC was 14.70 µg·h/ml after single dose oral administration of marbofloxacin (5 mg/kg) in broiler chickens. Value obtained in this study was nearly similar to 15.11 µg·h/ml [11] and 20.32 µg·h/ml [9] as reported in chicken. Contradictorily, higher values of AUC of 29.19 ± 9.12 µg·h/ml in cats were

found [14]. Comparatively, a lower value of 5.11 µg·h/ml was reported in horse by Bousquet-Melou *et al*. [6]. Lower value of AUC after repeated oral administration in broiler chickens as compared to cats may be due to physiological and anatomical distinctness among different species of animals and birds.

Table 2: Pharmacokinetic (PK) parameters of marbofloxacin following single dose oral administration (5 mg/kg b. wt.) in broiler chickens (n=8)

PK parameters	Unit	B1	B2	B3	B4	B5	B6	B7	B8	Mean ± SE
β	h-1	0.16	0.12	0.12	0.14	0.24	0.39	0.11	0.09	0.17 ± 0.03
$t_{½\beta}$	h	4.41	5.62	5.73	4.89	2.89	1.80	6.33	7.46	4.89 ± 0.65
$AUC_{0-\infty}$	µg·h/ml	14.71	15.32	25.71	27.74	6.20	4.68	11.47	11.75	14.70 ± 2.94
AUMC	$\mu g.h^2/ml$	98.37	120.41	216.39	210.17	24.61	17.80	118.67	131.54	117.25 ± 25.88
MRT	h	6.69	7.86	8.42	7.58	3.97	3.81	10.35	11.20	7.48 ± 0.94
$V_{d\ (area)}$	L/kg	2.16	2.65	1.61	1.27	3.36	2.77	3.98	4.58	2.80 ± 0.40
Cl_B	L/h/kg	0.34	0.33	0.19	0.18	0.81	1.07	0.44	0.43	0.47 ± 0.11

(β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; $AUC_{0-\infty}$: Area under curve; AUMC: Area under first moment of the plasma drug concentration; MRT: Mean Resident Time; $V_{d(area)}$: Apparent volume of distribution; $V_{d(ss)}$: Volume of distribution at steady state; Cl_B: Total body clearance)

The rate and extent of drug elimination vary among different species based on anatomical and physiological differences in the renal system. Fluoroquinolones are excreted by renal, biliary, or hepatic metabolic pathways. Renal tubular secretion is the major process involved in urinary excretion of drugs. Marbofloxacin is excreted as an unchanged form in the urine up to 40% of the total administered drug in dogs [11] and 30% in broiler chickens [8]. In the present study, the drug clearance (Cl_B) value was found to be 0.47 L/h/kg following oral administration of marbofloxacin in broiler chickens. However, comparatively lower Cl_B value was previously reported in chickens as 0.20 L/h/kg [12].

Mean Residence Time (MRT) value indicates the transient time through the body reflecting drug's persistence in the body. Thus, MRT becomes an important parameter to describe the length of drug persistence in the body. The mean MRT value following single dose oral administration of marbofloxacin was found to be 7.48 ± 0.94 h in the present study. A similar value of MRT was previously reported in chickens (7.03 ± 0.33 h) [12] whereas, relatively higher MRT value of 13.98 ± 0.37 h was also observed in chickens [9]. Similar MRT value of 8.97 h was reported in horse following a dose of 2 mg/kg by Bousquet-Melou *et al*. [6].

4. Conclusions

Following single dose oral administration of marbofloxacin (5.0 mg/kg) in broiler chickens, the mean ± SE value of plasma drug concentration of 0.41 ± 0.10 µg/ml was detected up to 12 h. The drug concentrations of marbofloxacin after single oral administration (5.0 mg/kg) were maintained above therapeutic concentration up to 12 hours post drug administration, which were larger than the reported concentration range of 0.125-0.20 µg/ml (MIC_{90}) against *E. coli* and other Gram-negative bacteria isolated from birds. In present study, long elimination half-life ($t_{½\beta}$), large volume of distribution (V_d) and area under curve (AUC) were observed after single oral administration of marbofloxacin in broiler chickens. These desirable pharmacokinetic profiles suggested that drug has adequacy to clear the deep seated bacterial infections in broiler chickens.

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