

Pharmacokinetics of ciprofloxacin in Nile tilapia, *Oreochromis niloticus*

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Abstract

Ciprofloxacin is a commonly used antibiotic in Bangladeshi aquaculture. However, the pharmacokinetic profile of ciprofloxacin in fish is not well studied. This study investigated the pharmacokinetics of ciprofloxacin in Nile tilapia (*Oreochromis niloticus*) following a single intraperitoneal administration at a dose of 50 mg/kg body weight. Blood samples from the fish were collected at 10 min and 1.5, 2.5, 5.0, and 6.0 h post administration. Serum ciprofloxacin concentrations were quantified using UFLC–MS/MS. Pharmacokinetic parameters were estimated using a non-compartmental analysis approach. The maximum serum concentration (C_{max}) of $28.60 \pm 2.97 \mu\text{g/mL}$ was reached at a T_{max} of 10 min, which indicates rapid systemic absorption. In the present study, ciprofloxacin exhibited a moderate elimination phase, with an elimination rate constant (k) of $0.34 \pm 0.01 \text{ h}^{-1}$ and a half-life ($T_{1/2}$) of $2.03 \pm 0.08 \text{ h}$. Moreover, the area under the concentration–time curve (AUC_i) was $77.80 \pm 9.00 \mu\text{g}\cdot\text{h/mL}$, and the extrapolated AUC_i was $88.90 \pm 10.50 \mu\text{g}\cdot\text{h/mL}$. The clearance (CL) of ciprofloxacin was $0.57 \pm 0.06 \text{ L/h}$, and the volume of distribution (V_d) was $1.66 \pm 0.15 \text{ L}$. From these findings, it can be suggested that ciprofloxacin demonstrates rapid absorption, adequate distribution, and efficient systemic clearance in Nile tilapia, supporting its therapeutic use in aquaculture. However, proper dosing and strict regulation of withdrawal periods are essential to minimize the risk of antimicrobial resistance.

Introduction

The aquaculture industry covers a wide range of species and production techniques, from simple traditional systems with little or no external inputs to intensive, high-density, and high-input production systems. Global aquaculture production has been steadily

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increasing, primarily through the intensification of production processes, which places substantial stress on animals and facilitates the emergence of infectious diseases that cause significant losses in aquaculture (Naylor et al., 2021; FAO, 2022). Moreover, the introduction of new culture species, improper culture management, and environmental changes also contribute to the emergence of new diseases. Unlike other animal production systems, disease management is more challenging because different animal species are often housed in the same water body, making them susceptible to infection by the same pathogens (Jones et al., 2015).

The use of therapeutics is a common and convenient technique for disease control in aquatic species (Mishra et al., 2023). Among the therapeutants, antibiotics are the most common and are enormously used in aquaculture (Chen et al., 2020). Ciprofloxacin (CIP) is a widely used antibiotic in intensive aquaculture to control disease outbreaks. The minimum inhibitory concentrations (MIC) of CIP are 0.01/0.02 µg/ml for *Aeromonas* and 0.25-0.5 µg/ml for *Pseudomonas* (Nouws et al., 1988). The efficiency of CIP in achieving therapeutic concentrations in fish plasma has been demonstrated by Nouws et al. (1988). Administration of CIP therapeutically through feed or water, helps to restore fish health and prevent mortality due to diseases like motile aeromonad septicemia and streptococcosis (Aziz et al. 2021). However, improper dosing and indiscriminate antibiotic use contribute to antimicrobial resistance (AMR) (Caputo et al., 2023). Consequently, antimicrobial resistance genes (ARGs) can spread to cultured fish or the aquatic environment via horizontal gene transfer and ultimately reach humans. In parallel to ARGs, antibiotic residues from fish or fishery products can reach human through the food chain (Heuer et al., 2009). Therefore, the development of AMR poses risks to the aquaculture industry and to public health (Cabello, 2006; Miranda et al., 2013). In this context, the design of aquaculture therapeutics should focus on achieving maximum efficacy and minimizing the risk of AMR development.

The study of drug pharmacokinetic properties is widely used to establish optimal drug doses in fish (Manna et al., 2022; Mallik et al., 2023; Rairat et al., 2023). Pharmacokinetic data, including drug absorption, half-life, plasma drug concentrations, and elimination half-life, help determine the doses required to inhibit bacterial growth and prevent AMR development. However, due to differences in feeding habits, metabolism, and other environmental interactions with organism physiology, the pharmacokinetics of a drug vary substantially among aquaculture species (Manna et al., 2022; Pinto et al., 2023; Vasuntrarak, 2020).

Nile tilapia, a popular farmed fish, is valued for its adaptability and nutritional profile. However, bacterial infections in tilapia aquaculture are common and cause significant economic losses, underscoring the need for effective therapeutics (Sherif & AbuLeila, 2022; Haenen et al., 2023). Although several studies have reported the pharmacokinetics of various antibiotics, pharmacokinetic data for CIP in Nile tilapia are scarce (Teles et al., 2016; Vasuntrarak, 2020; Corum et al., 2022, 2024). Therefore, we aimed to investigate the

pharmacokinetics of commercially available ciprofloxacin following an intraperitoneal administration in Nile tilapia (*O. niloticus*) reared in experimental tanks. The study will provide PK data to establish CIP doses for aquaculture practice.

Materials and Methods

Experimental animal: Nile tilapia (*Oreochromis niloticus*) weighing 150.83 ± 7.60 g (mean $\pm$ SD; $n = 12$, where 6 for control and 6 for treatment) was used in this study. Fish were collected from a reputable hatchery in Noakhali and reared individually in 70 L tanks. Adequate aeration was provided to maintain optimal dissolved oxygen (6-8 ppm) during the experiment. The experiment was conducted at the Department of Fisheries, University of Dhaka, Bangladesh. The temperature of the experimental system was 30 °C.

Drug administration, sample collection, and preparation: Ciprofloxacin (Ciprocin-VetR, Square Pharmaceutical Ltd., Bangladesh) was used at a dose of 50 mg/kg body weight to study the pharmacokinetics. The drug was administered through intraperitoneal injection. For control, the same amount of saline water was administered. Following administration, blood samples were collected at 10 min, and 1.5, 2.5, 5.0, and 6.0 h from the same fish. After blood collection and complete clotting, samples were centrifuged at 5000 rpm for 5 min to separate serum. The separated serum was stored at -20°C until further analysis. The research proposal was approved by the ethics review committee of the Faculty of Biological Sciences, University of Dhaka, Bangladesh (ethics approval Ref. No. 210/Biol.Scs).

Determination of serum ciprofloxacin concentration: The serum samples were diluted 4 times with 5% trichloroacetic acid (TCA). The mixture was vortexed for 5 min and centrifuged at 3000 rpm for 20 min. The supernatant was filtered through a nylon filter (pore size = 0.45 μ m) and stored at -20°C until instrumental analysis. The analysis was performed at the Ehime University, Japan using Ultra-Fast Liquid Chromatography coupled with Tandem Mass Spectrometry (UFLC-MS/MS). Before analysis, the samples were further diluted 10-fold using 0.1% formic acid in water: methanol (8:2, v/v). The diluted fish serum samples were injected into a Prominence UFLC XR (Shimadzu, Kyoto, Japan) coupled to a Sciex X500R QTOF System (Sciex, Tokyo, Japan) operating in the electrospray ionization (ESI) positive mode with SWATH acquisition (data-independent-acquisition, DIA) method for the quantitation of ciprofloxacin. Chromatographic separation was achieved with an Ascentis Express C18 analytical column (2.7 μ m, 100 $\times$ 2.1 mm; Supelco, Bellefonte, USA) at a flow rate of 0.25 mL/min. The mobile phase was 0.1% (v/v) formic acid in Milli-Q water (phase A) and 0.1% (v/v) formic acid in methanol (phase B). The gradient started at 2% B, increased to 60% B over 12 min, then gradually increased to 95% B over 3 min, remained at 95% B for 3 min, returned to 2% B over 0.5 min, and remained at 2% B for 4.5 min. The peak area of TOF-MS chromatograms for the protonated ion $[M+H]^+$ (m/z 332.1405 $\pm$ 0.005 Da) was used for quantitation (Figure 1a, 1d & 1g), while the TOF-MS isotopic pattern (Figure 1b, 1e & 1h) and TOF-MS/MS fragmentation were utilized for qualification (Figure 1c, 1f & 1i). Concentrations of ciprofloxacin were determined using the

absolute calibration curve method. The limits of detection (LODs) and limits of quantification (LOQs) for ciprofloxacin were determined using the signal-to-noise method. Concentrations corresponding to signal-to-noise ratios of 3 and 10 were defined as LOD and LOQ, respectively. The calculated LOD and LOQ were 2.6 and 8.8 ng/mL serum, respectively.

Pharmacokinetic analysis: The pharmacokinetic analysis was performed as described by Rairat *et al.* (2020). The Pharmacokinetic (PK) parameters included volume of distribution, elimination half-life ($T_{1/2}$), area under the serum concentration-time curve (AUC), maximum serum concentration (C_{max}), and time of maximum concentration (T_{max}), and mean residence time (MRT), which were analyzed by the non-compartmental model using PKSolver 2.0 software (China Pharmaceutical University) (Zhang *et al.*, 2010).

Statistical analysis: The PK parameters were calculated using PKSolver 2.0 software. The data were initially calculated using Microsoft Excel 2016 software. Mean and standard deviation (SD) were calculated from the six values ($n = 6$) of the treatment group.

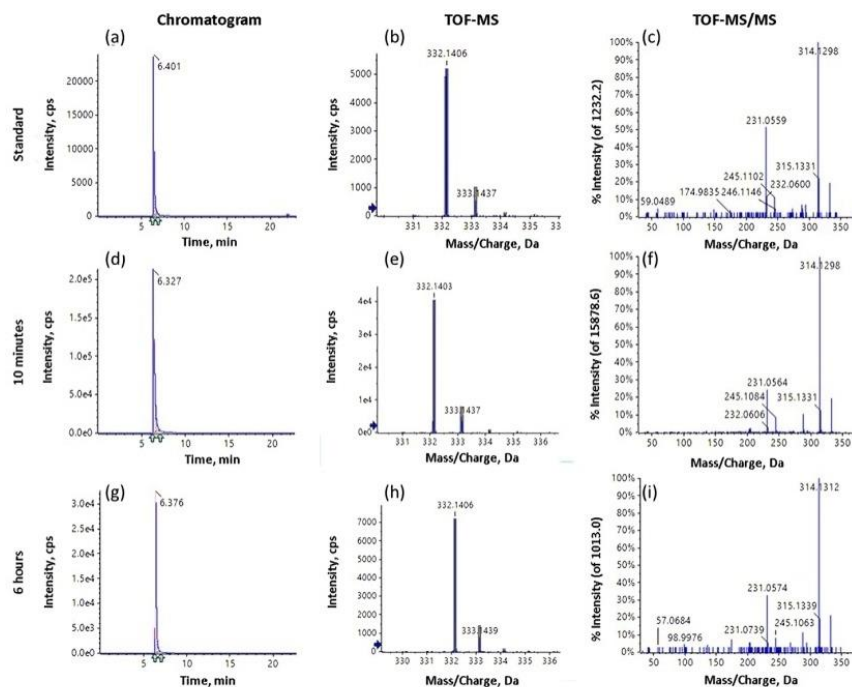


Fig. 1. Chromatogram of ciprofloxacin (CIP) quantification and qualification. CIP standard 50 ppb (a), CIP in serum (10-fold dilution) of tilapia after 10 min (d) & 6 h (g) samples; TOF-MS isotopic pattern of 50 ppb standard (b), CIP in samples of 10 min (e) & 6 h (h); and TOF-MS/MS fragmentation of 50 ppb standard (c), CIP in samples of 10 min (f) & 6 h (i).

Results and Discussion

The pharmacokinetics of ciprofloxacin in Nile tilapia was analyzed using key pharmacokinetic parameters (Table 1). The maximum serum concentration (C_{max}) was $28.60 \pm 2.97 \mu\text{g/mL}$ at a time (T_{max}) of 10 min, indicating rapid absorption after intraperitoneal administration. The serum concentration decreased steadily, with the last observed concentration (C_{last}) of $3.85 \pm 0.57 \mu\text{g/mL}$ at $T_{last} = 6.00 \pm 0.00 \text{ h}$ (mean $\pm$ SD; $n = 6$). However, no ciprofloxacin was detected in the control fish.

The area under the curve for the observed period (AUC_t) was $77.80 \pm 9.00 \mu\text{g}\cdot\text{h/mL}$, while the total drug exposure extrapolated to infinity (AUC_i) was $88.90 \pm 10.50 \mu\text{g}\cdot\text{h/mL}$. The area under the first moment curve ($AUMC_t$) was $163.10 \pm 20.10 \mu\text{g}\cdot\text{h}^2/\text{mL}$, and the extrapolated $AUMC_i$ was $262.90 \pm 33.90 \mu\text{g}\cdot\text{h}^2/\text{mL}$, indicating the time-weighted drug exposure.

The mean residence time during the observation period (MRT_t) was $2.09 \pm 0.03 \text{ h}$, extending to $2.95 \pm 0.05 \text{ h}$ when extrapolated to infinity (MRT_i) (Table 1). The present study found the elimination rate constant (k) to be $0.34 \pm 0.01/\text{h}$, with a corresponding half-life ($T_{1/2}$) of $2.03 \pm 0.08 \text{ h}$. The clearance (CL) of ciprofloxacin was calculated as $0.57 \pm 0.06 \text{ L/h}$, while the volume of distribution (V_d) was $1.66 \pm 0.15 \text{ L}$, indicating extensive tissue penetration. Pre-administration metrics, such as $AUCM_{i_pre} = 269.60 \pm 38.20 \mu\text{g}\cdot\text{h}^2/\text{mL}$ and $V_{d_pre} = 1.65 \pm 0.15 \text{ L}$, were also included to refine the calculations (Table 1).

In the present study, the serum concentration of ciprofloxacin rose sharply, ultimately reaching a maximum (C_{max}) of $28.60 \pm 2.97 \mu\text{g/mL}$ at approximately 10 minutes. The serum concentration then decreased steadily, which indicated its elimination phase (Figure 2a). Using the observed concentration data, the serum concentration-time profile of ciprofloxacin was calculated to interpret the potential elimination pattern (Figure 2a). The extrapolated line closely follows the observed data in the elimination phase, indicating a good fit for modeling purposes (Figure 2b). Figure 2b shows the complete elimination kinetics of ciprofloxacin, particularly when concentrations fall below detectable limits in serum. The extrapolated data showed that ciprofloxacin in tilapia serum could be completely eliminated within 36-48 h.

Table 1. Pharmacokinetic profile of ciprofloxacin following a single intraperitoneal injection of 50 mg/kg in Nile tilapia. No ciprofloxacin was detected in the control samples

Pharmacokinetic parameter	Description	Value (Mean $\pm$ SD) (n = 6)
C_{max} ($\mu\text{g/mL}$)	Maximum concentration	28.60 ± 2.97
T_{max} (min)	Time at which maximum serum concentration was reached	10 ± 0.00
C_{last} ($\mu\text{g/mL}$)	Last measurable serum concentration of the drug	3.85 ± 0.57
T_{last} (h)	Time at which the last measurable serum concentration is observed	6.00 ± 0.00
AUC_t ($\mu\text{g}\cdot\text{h/mL}$)	Area under the concentration-time curve	77.80 ± 9.00
$AUMC_t$ ($\mu\text{g}\cdot\text{h}^2/\text{mL}$)	Area under the first moment curve up to the last measurable concentration, indicating time-weighted drug exposure	163.10 ± 20.10
MRT_t (h)	Mean residence time for the observed data	2.09 ± 0.03
k (1/h)	Elimination rate constant	0.34 ± 0.01
$T_{1/2}$ (h)	Half-life	2.03 ± 0.08
AUC_i ($\mu\text{g}\cdot\text{h/mL}$)	Total AUC extrapolated to infinity, representing overall drug exposure	88.90 ± 10.50
$AUMC_i$ ($\mu\text{g}\cdot\text{h}^2/\text{mL}$)	Total AUMC extrapolated to infinity	262.90 ± 33.90
MRT_i (h)	Mean residence time extrapolated to infinity	2.95 ± 0.05
CL (L/h)	Clearance, the volume of serum cleared of the drug per unit time	0.57 ± 0.06
V_d (L)	Volume of distribution, indicating the extent of the drug's distribution into body tissues	1.66 ± 0.15
C_{last_pre} ($\mu\text{g/mL}$)	Last measurable serum concentration in pre-treatment condition	4.05 ± 0.53
AUC_{i_pre} ($\mu\text{g}\cdot\text{h/mL}$)	Total AUC extrapolated to infinity in pre-treatment condition	89.70 ± 10.90
$AUMC_{i_pre}$ ($\mu\text{g}\cdot\text{h}^2/\text{mL}$)	Area under the first moment curve extrapolated to infinity in pre-treatment condition	269.60 ± 38.20
MRT_{i_pre} (h)	MRT extrapolated to infinity in pre-treatment condition	3.00 ± 0.06
CL_{pre} (L/h)	Clearance in pre-treatment condition	0.56 ± 0.07
V_{d_pre} (L)	Volume of distribution in pre-treatment condition	1.65 ± 0.15

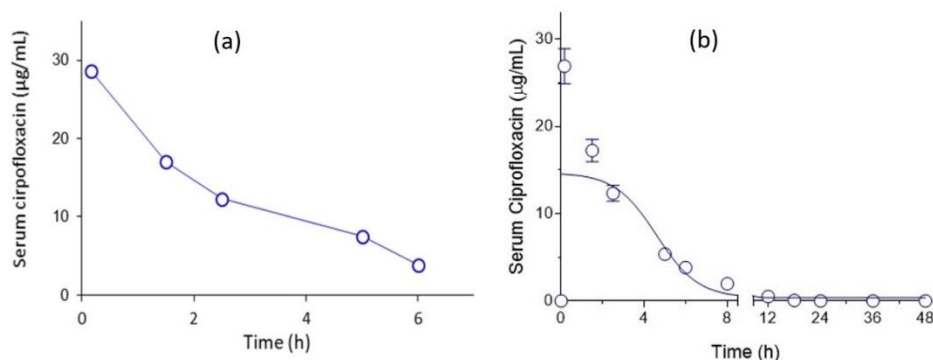


Fig. 2. Serum concentration-time profiles of ciprofloxacin (CIP) in Nile tilapia after intraperitoneal administration (50 mg-ciprofloxacin/kg of body weight). (a) Measured serum concentration ($\mu\text{g/mL}$), (b) Extrapolated serum concentration ($\mu\text{g/mL}$)-time profile to estimate the terminal elimination of ciprofloxacin from tilapia.

The present study demonstrates the rapid absorption and moderate elimination of ciprofloxacin in Nile tilapia, indicating the suitability for controlling bacterial infections in aquaculture. The high serum concentration ($C_{\text{max}} = 28.60 \mu\text{g/mL}$) and short T_{max} (10 minutes) were observed following intraperitoneal injection, indicating rapid systemic absorption of ciprofloxacin in Nile tilapia in the current study. Previously, different PK parameters ($C_{\text{max}} = 1.24 \pm 0.37 \mu\text{g/mL}$; $T_{\text{max}} = 8 \text{ h}$; $T_{1/2} = 19.36 \text{ h}$) for enrofloxacin were observed in Nile tilapia following oral administration (Teles et al., 2016). Similarly, a lower serum C_{max} ($3.59 \mu\text{g/mL}$) of enrofloxacin has also been reported in yellow croaker upon a single oral dose at 10 mg/kg, with ciprofloxacin levels reaching a peak ($0.55 \mu\text{g/mL}$) at 24 h after administration (Ma et al., 2022). In another study on enrofloxacin in turbot following oral administration (10 mg/kg) at 16 °C, a slower absorption rate ($T_{\text{max}} = 4.00 \text{ hours}$) and peak concentration ($C_{\text{max}} = 5.54 \mu\text{g/mL}$) were observed (Liang et al., 2012). A lower ciprofloxacin concentration was observed in hybrid tilapia serum ($2.25 \pm 0.87 \mu\text{g/mL}$) after 8 hours of bath exposure to 50 mg/L, as determined by enzyme-linked immunosorbent assay (ELISA) (Okorpafor et al., 2016).

Compared with many published studies, the serum peak concentration of ciprofloxacin observed in this study occurred earlier. However, most literature on 'ciprofloxacin' in aquatic species has measured ciprofloxacin as a metabolite after enrofloxacin administration, which is not directly comparable to the present study in which ciprofloxacin itself was administered intraperitoneally. The key comparator issue is drug identity and route. In Nile tilapia, Teles et al. (2016) a C_{max} of $1.24 \mu\text{g/mL}$, T_{max} of 8 h after oral enrofloxacin administration, and only limited ciprofloxacin formation (around 1.3% conversion) were reported, which is consistent with a delayed metabolite profile rather than direct ciprofloxacin absorption (Teles et al., 2016). Similarly, Fang et al. (2007) found that

after oral enrofloxacin in mud crab, enrofloxacin peaked in hemolymph at 2 h, while ciprofloxacin peaked much later (48 h). In Yellow River carp, Yang *et al.* (2022) described oral enrofloxacin showing slow absorption and noted only trace ciprofloxacin in some samples. By contrast, oral enrofloxacin absorption half-life in turbot was around 2 h at 16–10 °C, and classic fish ciprofloxacin work by Nouws *et al.* (1988) already documented species-dependent ciprofloxacin pharmacokinetics in carp, African catfish and rainbow trout. Across aquatic PK studies, route effects have been documented that are strong and consistent. In Nile tilapia, Corum *et al.* (2022) compared intravascular, intraperitoneal, and oral enrofloxacin administration, and showed route-dependent differences in plasma and muscle disposition. In crucian carp, Shan *et al.* (2018) reported enrofloxacin T_{max} 4 h after IM, 8 h after oral, and 4 h after bath exposure, with IM also giving the highest AUC . Similarly, major pharmacokinetic differences among IM, oral, bath, and IV bath exposures in red pacu work by Lewbart *et al.* (1997) and European cuttlefish work by Gore *et al.* (2005). Accordingly, the rapid T_{max} observed in this study is best explained using an intraperitoneally administered solution, which avoids feed heterogeneity, gastrointestinal transit, and dissolution limitations that slow oral absorption. Taken together, the rapid serum appearance of ciprofloxacin is best interpreted as the combination of direct ciprofloxacin dosing, intraperitoneal administration, and 30 °C warm-water conditions, rather than as a contradiction of the literature.

The moderate half-life of ciprofloxacin (2.03 ± 0.08 h) suggests that the dosing interval was sufficient to maintain therapeutic serum levels, thereby minimizing the risk of toxicity. In carp, African catfish, and trout, a similar half-life of ciprofloxacin was reported upon intravenous injection (Nouws *et al.*, 1988). However, Stoffregen *et al.* (1997) enrofloxacin showed a higher half-life ($T_{1/2} = 32.32$ h) in juvenile Atlantic salmon following intraperitoneal injection. The high volume of distribution ($V_d = 1.66 \pm 0.15$ L/kg) observed in the present study indicates extensive tissue penetration, supporting the effectiveness of ciprofloxacin against systemic bacterial infections. A similar volume of distribution (1.47 L/kg) of enrofloxacin has been reported in yellow croaker by Ma *et al.* (2022). However, Nouws *et al.* (1988) showed lower V_d values of ciprofloxacin in carp, African catfish, and trout. In another study, enrofloxacin in turbot exhibited prolonged elimination ($T_{1/2} = 98.63$ hours) and a lower V_d of 3.96 L/kg upon oral administration at 10mg/kg, reflecting slower metabolism and limited distribution (Liang *et al.*, 2012).

In the present study, the area under the concentration-time curve (AUC) value of ciprofloxacin was 77.80 ± 9.00 $\mu\text{g}\cdot\text{h/mL}$, which is similar to the finding of Stoffregen *et al.* (1997) who showed an AUC value around 75 $\mu\text{g}\cdot\text{h/mL}$ of enrofloxacin in juvenile Atlantic salmon using intraperitoneal injection. On the other hand, Nouws *et al.* (1988) reported different AUC values of ciprofloxacin in carp, African catfish, and rainbow trout. They reported the AUC values of 104.00 ± 11.20 , 55.20 ± 1.50 , and 53.00 ± 6.70 $\mu\text{g}\cdot\text{h/mL}$, in carp, African catfish, and trout, respectively. Higher AUC (155.24 $\mu\text{g}\cdot\text{h/mL}$) of enrofloxacin has also been reported in yellow croaker by Ma *et al.* (2022). In a previous study, a higher AUC

for ciprofloxacin was also reported in turbot ($424.45 \mu\text{g}\cdot\text{h}/\text{mL}$), reflecting prolonged drug exposure and slower elimination. A high AUC indicates prolonged or enhanced presence of the drug in the systemic circulation, which has important implications for its therapeutic and toxic effects. The AUC observed in the present study confirms sufficient systemic exposure to achieve therapeutic efficacy. The clearance rate of ciprofloxacin ($CL = 0.57 \pm 0.06 \text{ L/h}$) was higher compared to previous findings in a study on enrofloxacin in yellow croaker, where a lower clearance ($CL = 0.06 \text{ L/h}$) was reported by Ma et al. (2022). The higher clearance rate indicates efficient elimination, reducing the likelihood of residue accumulation in edible tissues. During the 48 h extrapolation, the expected concentration of ciprofloxacin in the bloodstream exhibited a linear elimination pattern, and at 48 h, no serum ciprofloxacin was detected, suggesting complete clearance.

The pharmacokinetics of ciprofloxacin observed in Nile tilapia in this study showed species-specific variation compared with previous studies in other fish species, including common carp, African catfish, yellow croaker, Atlantic salmon, and Rainbow trout. Species-specific variations in the pharmacokinetics of antibiotics in fish may stem from differences in metabolism, digestive physiology, gut microbiota, temperature-dependent enzymatic activity, and feeding or social behaviors (Toutain et al., 2010).

Conclusion

The pharmacokinetics of ciprofloxacin in Nile tilapia demonstrate rapid absorption, moderate distribution, and a steady elimination phase. The clearance and volume of distribution values suggest that the drug is efficiently distributed and eliminated from the system. The findings support the suitability of ciprofloxacin for therapeutic use in aquaculture. However, ciprofloxacin must be used carefully to mitigate the risks of AMR and environmental contamination. Adherence to withdrawal periods and proper dosing are critical to ensuring consumer safety for fish and fish products. Future studies on pharmacokinetics under varying environmental conditions (e.g., temperature, salinity) should be conducted to account for factors that influence drug metabolism in aquaculture species. Species-specific studies should be conducted to further refine dosing regimens for aquaculture species. Furthermore, species and drug-specific research is necessary to ensure the judicious and effective use of antibiotics, thereby improving animal welfare, mitigating environmental impacts, and maintaining the safety of animal-derived products for human consumption.

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Authors' contribution

Shankar Chandra Mandal: study design, data acquisition, fund acquisition, and drafting manuscript, **Md. Shafiujjaman, Asif Rahman:** performed the experiment, and editing of the manuscript, **Shimul Halder:** study design, data analysis, critical review, and editing of the manuscript, **Goutam Kumar Kundu:** study design, critical review, and editing of the manuscript, **Joya Boidya:** data analysis, critical review, and editing of the manuscript. **Rumi Tanoue, Tatsuya Kunisue:** performed method development and validation, critical review and editing of the manuscript, **Kozo Watanabe:** study design, critical review and editing of the manuscript, **Anwar Hossain:** study design, data acquisition, critical review and editing of the manuscript. All authors read and approved the manuscript.

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