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Pharmacokinetics of Enrofloxacin in Rohu (*Labeo rohita*): A Comparison of Bath, Intramuscular, and Oral Administration

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Authors' contributions

This work was carried out in collaboration among all authors. Author AS performed the work as part of his PG research work, literature review, data collection, conceptualization and designed the study. Author LK did the conception and design of the study, supervision, data curation and visualization, revising draft critically for intellectual content. Author NH analysed and drafted the manuscript. Author ES done the data curation and visualization. Author SAS supervised the findings of this work. Author PS did the investigation, editing and supervision and the final approval of the version to be published. All authors read and approved the final manuscript.

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ABSTRACT

Enrofloxacin, fluoroquinolone antibacterial drug is widely used in aquaculture for the control of infectious diseases. Understanding the pharmacokinetics of enrofloxacin in *Labeo rohita* is critical for developing optimized therapeutic strategies so as to maximize efficacy and to minimize the emergence of resistant pathogens. Hence, the present study was aimed at studying the pharmacokinetics of enroflocacin in rohu fish (*Labeo rohita).* The pharmacokinetics of enrofloxacin was studied after single intramuscular or oral administration at 10 mg/kg body weight and following bath exposure at the concentration of 5 mg/L for 5 h. Blood samples were collected at predetermined time intervals. The plasma concentrations of enrofloxacin were determined by high performance liquid chromatography. The pharmacokinetic parameters were analyzed using a non-compartmental method. Cmax and Tmax varied depending on the route of administration, with intramuscular administration resulting in highest plasma concentration in short Tmax indicating rapid and better absorption. Similarly, $AUC_{0-\infty}$ was found to be highest (166.51 μg.h.mL^{-1}) following intramuscular administration indicating higher total systemic drug exposure compared to oral and bath exposure. Elimination half-life ($t_{1/2}$) was found to be 43.31, 34.65, and 21.66 h following intramuscular, oral, and bath exposure, respectively. Intramuscular administration provided the highest plasma concentrations and the longest elimination half-life, suggesting its suitability for sustained treatment efficacy. The volume of distribution (Vd/F) was found to be maximum after oral administration (6.52 L/kg) as compared to the intramuscular route (3.75 L/kg) and bath exposure (2.07 L/kg). The results of our study indicate that enrofloxacin was well absorbed, widely distributed, and slowly eliminated in rohu following all three routes of administration. Our results suggest that enrofloxacin administered intramuscularly (10 mg/kg), orally (10 mg/kg), or by bath immersion (5 mg/L for 5 h) achieves sufficient plasma concentrations to treat pathogens with MIC ≤ 0.3 μg/mL.

Keywords: Enrofloxacin; non-compartmental method; pharmacokinetics; Labeo rohita; bath immersion.

1. INTRODUCTION

Aquaculture has been one of the fastest-growing industries, contributing nearly 46 % of global fish production in 2018. India ranks second in world inland fish production, with a total production of 1.7 million tonnes in 2018, around 14% of global fish production (FAO, 2020). Indian fisheries have expanded by more than 13 times over the past 60 years, with fish production rising from 0.75 million tonnes in 1950–51 to 14.16 million tonnes in 2019–20. (MoFAHD, 2020). The three major carps, namely, Catla (Catla catla), rohu (Labeo rohita), and Mrigal (*Cirrhinus mirgala*), contribute around 70-75% of total aquaculture production in India (Jayasankar, 2018). Among these, Labeo rohita has been the most demandable and extensively cultured species in Indian subcontinents. With the increasing demand and intensification of production, an outbreak of diseases remains a major constraint. Bacterial infections are most common due to various factors such as high stocking density, stress, etc. Hence, antibacterials are used in aquaculture as prophylaxis and for treating bacterial infections in fish.

Enrofloxacin, a fluoroquinolone antibacterial drug, was developed exclusively for use in veterinary medicine. It has a broad spectrum of antibacterial activity, including Gram-negative and Gram-positive bacteria, as well as Mycoplasma spp. It has been widely used in fish due to its properties, such as good absorption, large volume of distribution, high bioavailability, and long terminal half-life. It is effective against Aeromonas spp., Vibrio spp., and Yersinia spp. and serves as a last-line treatment in aquaculture for bacterial infections caused by F. columnare, A. hydrophila, A. sobria, etc.

The effective control of infections depends on the choice of the drug and correct treatment regimen. Optimization of therapeutic regimen is the key to maximize the efficacy and to minimize the emergence of drug-resistant pathogens. In general, dose of a drug is optimized by integrating pharmacokinetic properties of the drug with its pharmacodynamic data. The pharmacokinetics of a drug is species-dependent and it was impossible to extrapolate pharmacokinetic data even for closely related species. Hence, the dosage schedule must be optimized based on the pharmacokinetic data generated for the particular species (Rairat et al, 2024).

The pharmacokinetics of enrofloxacin have been studied in fish species such as yellow cat fish (Jia et al., 2924), northern snake head fish (Zhang et al., 2023), Nile tilapia (Corum et al., 2022), crucian carp (Shan et al., 2018), snakehead fish (Fang et al., 2016), rainbow trout (Urzua et al., 2020; Lucchetti et al.,2004), large yellow croaker (Ma et al., 2022), red pacu (Lewbart et al., 1997), and largemouth bass (Shan et al., 2020). However, there were no reports on the pharmacokinetics of enrofloxacin in rohu. Hence the present study was undertaken to study the pharmacokinetics of enrofloxacin in rohu following three routes of administration, viz., intramuscular, oral, and bath immersion. Comparison of pharmacokinetics following different routes of exposure will provide insights into the differences in the drug pharmacokinetics for optimization of drug delivery for effective control of infections in aquaculture.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The enrofloxacin analytical standard was purchased from M/s Sigma Chemicals in Bangalore. Pure enrofloxacin obtained as gratis from M/s Intas Pharmaceuticals, Ahmedabad, was used in this study. HPLC grade acetonitrile was purchased from M/s Thermo Fisher Scientific India Pvt., Ltd., Mumbai. All other chemicals and reagents used in the study are analytical grade.

2.2 Experimental Fish

Two hundred and sixteen healthy rohu (Labeo rohita) with an average weight of 250 g were collected from M/s Rhea fish farm and hatchery unit, Janapanchatram, Chennai. The fish were kept in aquarium tanks of 160 L capacity and fed with drug-free pelleted feed. They were maintained in well-aerated water of 0.5 ppt salinity with a pH of 7.5. The water temperature in the fish tank was maintained at 18.0 – 20.5 °C. The water in the fish tanks was changed every two days. Fish were acclimatized to the laboratory conditions before the experiment for two weeks.

2.3 Pharmacokinetic Trial

A total of 216 fish were randomly divided into three groups and maintained in an aquarium tank of 160 L capacity containing 150 L water. Enrofloxacin was administered at 10 mg/kg body weight for the intramuscular and oral trials. Intramuscular injections were given below the dorsal fin and above the lateral line, while oral administration was done using an oral gavage. Enrofloxacin solution was added to 150 L of water for bath administration to achieve a final concentration of 5 mg/L. Fishes were immersed in medicated water for 5 h and then transferred to a drug-free freshwater tank for 91 h.

Blood samples (0.5 mL) were collected in heparinized tubes from the tail sinus before drug administration and at 15 min, 30 min, 1, 2, 4, 8, 12, 24, 48, 72, and 96 h after drug administration. Fish were anaesthetized by immersing in the water containing clove oil (0.05 mL per 500 mL of water) before blood collection. Blood samples were collected from six fishes at each time point. Plasma was separated by centrifugation at 5000 rpm for 5 min and stored at -20ºC until further analysis.

2.4 Analysis of Enrofloxacin Concentration

Plasma enrofloxacin concentrations were determined using High-Performance Liquid Chromatography (Waters, USA). The HPLC system consisted of a pump (Model 515), rheodyne manual injector with 20 µL loop, pump control module (Model PC2), column oven, Temperature control module (TC2), UV-Vis Detector (Model 2489) and Empower 2® software for analysis. The chromatographic separation was done using Synchronis C18 column (250 mm x 4.6 mm, 5 µm) purchased from M/s Thermo-Fisher Scientific, USA. For analysis of enrofloxacin concentration in plasma, the chromatographic method of Kalaiselvi et al. (2006) was followed with modifications. The mobile phase consisted of 0.1% Orthophosphoric acid (pH 2.5 adjusted with 45% KOH): Acetonitrile in the ratio of 70:30. The flow rate of the mobile phase was kept at 1.0 mL/min and the column temperature was maintained at 27ºC. The detection wavelength was 278 nm, and the run time of the samples was 10 min.

A liquid-liquid extraction procedure was followed to extract enrofloxacin from fish plasma. To 200 µL of plasma or spiked plasma, 400 µL of acetonitrile (1:2) was added, mixed, vortexed, and centrifuged at 10000 rpm for 5 minutes. After centrifugation, the supernatant was collected, and deionized water was added in a 1:1 ratio. The supernatant was filtered through a 0.22 µ

HNN membrane filter and 20 µL was injected into the HPLC system for analysis.

2.5 Pharmacokinetic Analysis

The mean plasma concentration-time data of enrofloxacin was used for pharmacokinetic analysis. Non-compartmental techniques were used to analyze the pharmacokinetic parameters. The area under the concentration-time curve (AUC) and area under the moment curve (AUMC) were determined by the trapezoidal method and extrapolated to infinity using the last measured concentration (C_{last}) and slope (β). Terminal slope (β) was determined by linear regression analysis of the drug concentration in the elimination phase of the concentration versus time curve. The mean residence time (MRT) was determined as the ratio of $AUMC_{0-\infty}$ to $AUC_{0-\infty}$. Maximal plasma concentration (C_{max}) and the time to reach maximal plasma concentration (T_{max}) were determined by observation of the data.

3. RESULTS AND DISCUSSION

The HPLC method standardized for the analysis of enrofloxacin was highly specific, with no interfering peak at the retention time of enrofloxacin. The method was linear in the range of 0.01 to 10 µg/mL and the recovery of enrofloxacin from the fish plasma was found to be 94.36%. The precision of the method, as determined by the intra-day and inter-day coefficient of variations at three different concentrations (0.01, 0.1, and 1.0 µg/mL), were found to be 3.08 and 8.52%, respectively.

Plasma enrofloxacin concentration versus the time curve following intramuscular, oral, and bath immersion are shown in Table 1 and Figs. 1, 2, and 3. Following the intramuscular route, the mean plasma enrofloxacin concentration at 15 min was 1.158 ± 0.132 µg/mL. The plasma concentration showed an increasing trend up to 4 h post-dose and declined thereafter. The maximum plasma concentration (C_{max}) was 5.602 ± 1.433 and was observed at 4 h (T_{max}). A detectable concentration of enrofloxacin was observed till the last sampling time (96 h).

Following oral administration, all the fishes showed detectable plasma concentration at 15 minutes post-dose. The plasma concentration gradually increased with time up to 8 and then declined. The maximum plasma concentration (C_{max}) of 3.380 ± 0.229 µg/mL was observed at 8 h (T_{max}). The detectable concentration of enrofloxacin was observed only in three fishes at the last sampling time point (96h).

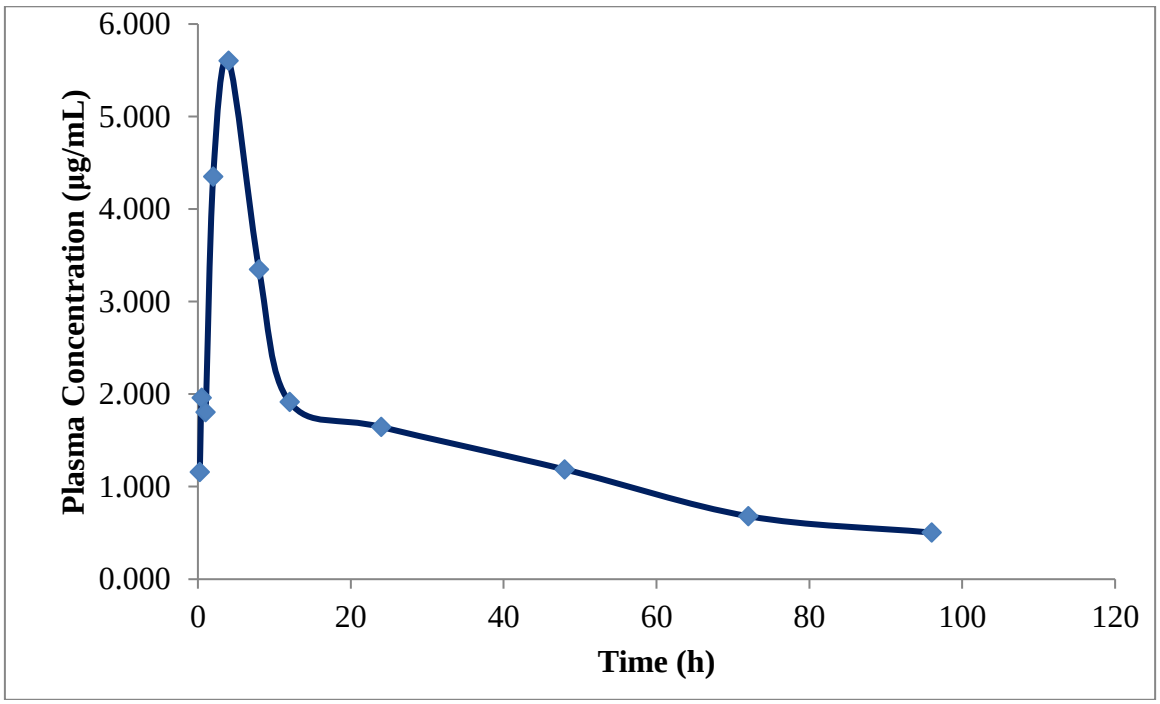

Fig. 1. Mean Plasma concentration-time curve of enrofloxacin after single intramuscular administration (10 mg/kg) in rohu fish

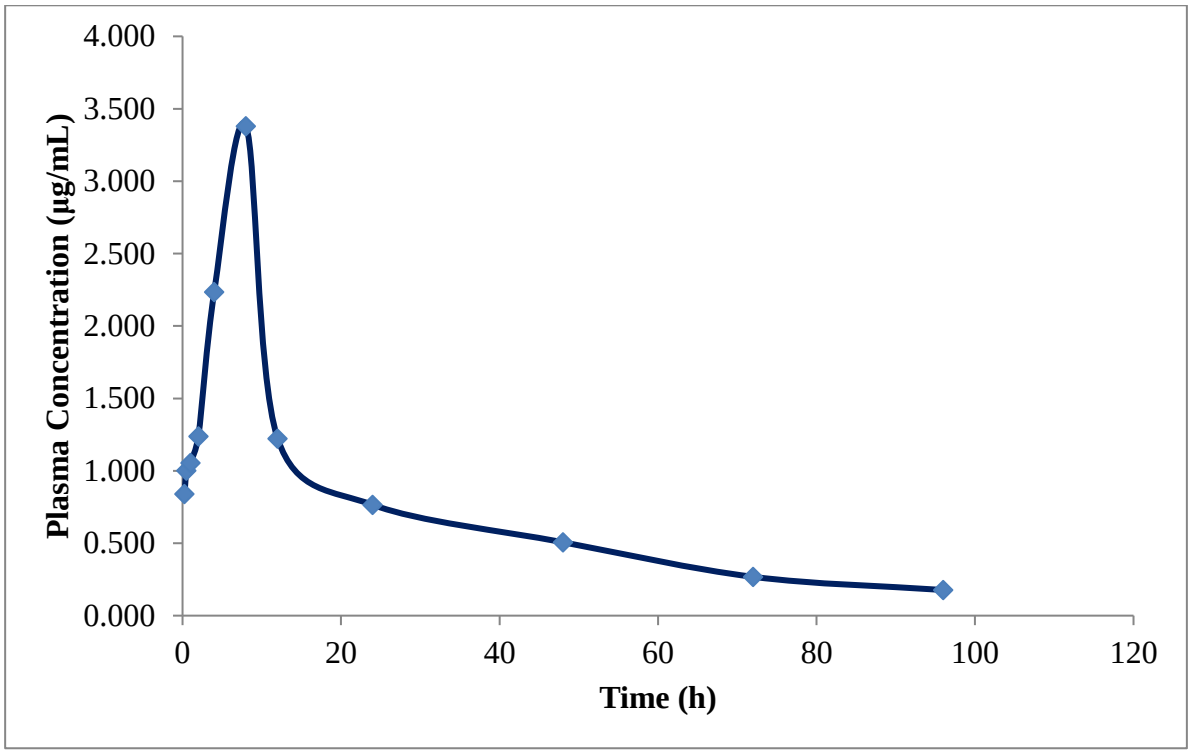

Fig. 2. Mean Plasma concentration-time curve of enrofloxacin after single oral administration (10 mg/kg) in rohu fish

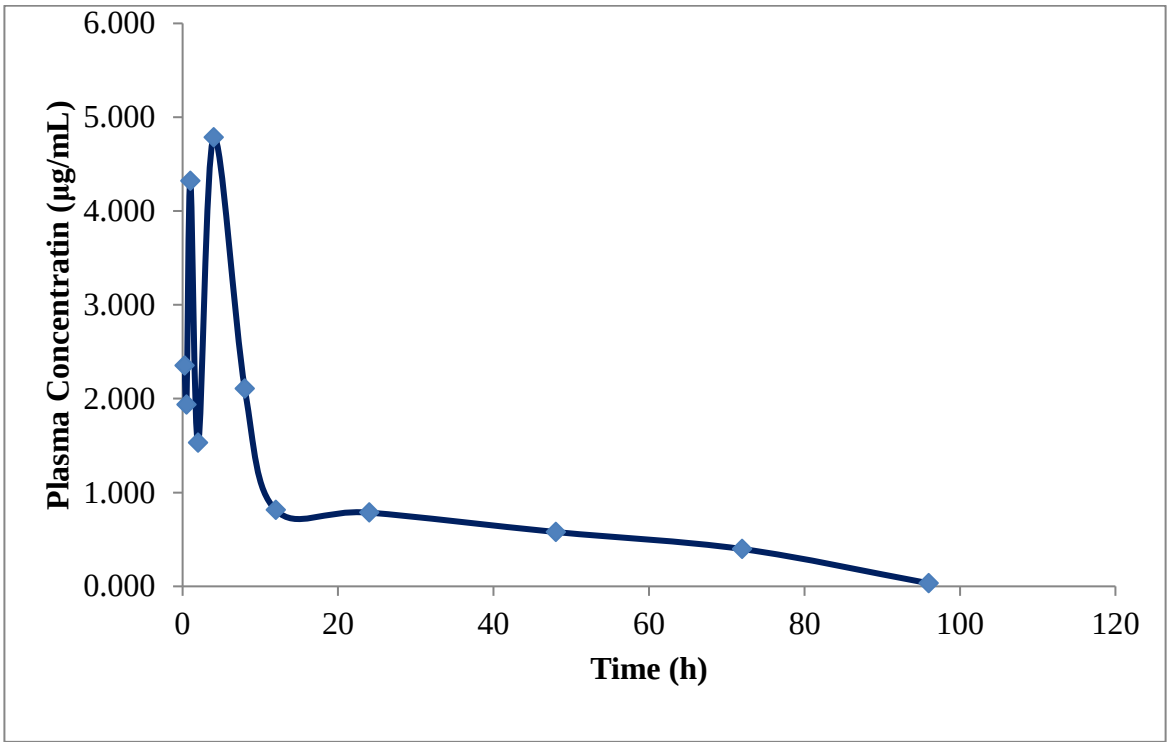

Fig. 3. Mean Plasma concentration-time curve of enrofloxacin after bath administration immersion (5 mg/L for 5 h) in rohu fish

Table 1. Comparison of mean Plasma concentration of enrofloxacin after administration by three routes – intramuscular, oral, bath exposure

Time (h)	Mean Plasma concentration (µg/mL)		
	Intramuscular	Oral	Bath exposure
0.25	1.158	0.840	2.353
0.5	1.961	1.002	1.938
1.0	1.803	1.055	4.322
2	4.350	1.238	1.531
4	5.602	2.235	4.785
8	3.347	3.380	2.107
12	1.914	1.223	0.813
24	1.644	0.765	0.786
48	1.186	0.507	0.579
72	0.681	0.268	0.398
96	0.504	0.177	0.033

Table 2. Pharmacokinetic parameters of enrofloxacin following different routes of administration – intramuscular, oral and bath exposure

Parameter	Unit	Intramuscular	Oral	Bath exposure
Cmax	$\mu g.mL^{-1}$	5.60	3.38	4.79
Tmax	h	4	8	4
β	h^{-1}	0.02	0.02	0.03
$t_{1/2}$	h	43.31	34.65	21.66
AUC_{0-t}	$\mu g.h.mL^{-1}$	135.01	67.84	74.14
$AUC_{0-\infty}$	$\mu g.h.mL^{-1}$	166.51	76.69	75.45
$AUMC_{0-t}$	$\mu g.h^2.mL^{-1}$	4207.11	1838.63	1943.00
$AUMC_{0-\infty}$	$\mu g.h^2.mL^{-1}$	9199.86	3130.73	2074.22
MRT	h	55.25	40.82	27.49
Cl/F	$L.h^{-1}.Kg^{-1}$	0.06	0.13	0.07
VD/F	$L.Kg^{-1}$	3.75	6.52	2.07

Following bath exposure, all the fishes showed detectable plasma concentration at 15 min post-dose and there was a large variation in the initial plasma concentration among individual fishes, ranging from 0.302 to 3.696 µg/mL. The maximum mean plasma concentration of 4.785 ± 1.877 µg/mL was achieved at 4 h.

The pharmacokinetic parameters calculated using mean plasma concentration are given in Table 2. Following the intramuscular route of administration, elimination half-life ($t_{1/2}$), area under the curve ($AUC_{0-\infty}$), clearance (Cl/F), and volume of distribution (V_d/F) were 43.31 h, 166.51 $\mu g.h.mL^{-1}$, 0.06 $L.h^{-1}.Kg^{-1}$ and 3.75 $L.Kg^{-1}$, respectively. While after oral administration, the elimination half-life ($t_{1/2}$), area under the curve ($AUC_{0-\infty}$), clearance (Cl/F), and volume of distribution (V_d/F) were 34.65 h, 76.69 $\mu g.h.mL^{-1}$, 0.13 $L.h^{-1}.Kg^{-1}$ and 6.52 $L.Kg^{-1}$, respectively.

The elimination half-life of 21.66 h, $AUC_{0-\infty}$ of 75.45 $\mu g.h.mL^{-1}$, clearance of 0.07 $L.h^{-1}.Kg^{-1}$ and volume of distribution of 2.07 $L.Kg^{-1}$ were observed following bath exposure of enrofloxacin in rohu.

In the present study, enrofloxacin was detected in the plasma within 15 minutes of administration following oral, intramuscular, and bath exposure, and it was detected up to 96 hours in all fish following oral and intramuscular administration. After oral administration, the enrofloxacin was detected at 96 h in only three fishes. The results of the present study agree with Udomkusonsri et al. (2007), who reported the presence of enrofloxacin in plasma up to 96 h post-dosing following intramuscular, oral, and bath exposure.

In the present study, C_{max} was 5.60 µg/mL after intramuscular administration at 10 mg/kg body weight. Jia et al (2024) reported Cmax of 4.124 µg/mL in Yellow cat fish while Cmax of 4.59 µg/mL in Koi carp was reported by Udomkusonsri et al. (2007) at the same dose rate. In a similar study, C_{max} was reported to be 2.29 µg/mL in Crucian carp with the same dose rate (Shan et al., 2018). In comparison to the

previous studies, the C_{max} was found to be greater in the present study, which indicates better absorption of the drug.

The T_{max} in the present study was 4 h following intramuscular administration, similar to the Tmax value (4.08 h) reported by Shan et al. (2018) for crucian carp. Udomkusonsri et al. (2007) reported a lower T_{max} of 1 h in Koi carp with the same dose rate.

The area under the curve ($AUC_{0-\infty}$) in the present study was 166.51 $\mu g.h.mL^{-1}$after intramuscular route. The AUC values obtained in the present study were higher compared to the value reported by Jia et al (2024) in yellow cat fish (108.36 $\mu g.h.mL^{-1}$) and by Udomkusonsri et al. (2007) in Koi carp (82.7 $\mu g.h.mL^{-1}$) at the same dose rate. However, Shan et al. (2018) reported a higher AUC value of 223.46 $\mu g.h.mL^{-1}$ in crucian carp at the same dose rate. The Mean residence time (MRT) in the present study was 55.25 h following intramuscular administration and it was higher than the MRT value (23.8 h) reported in Koi carp with the same dose rate (Udomkusonsri et al., 2007 $\mu g.h.mL^{-1}$).

The Half-life ($t_{1/2}$) in the present study following intramuscular administration was found to be 43.31 h, which was considerably longer than the half-life (17.9 h) reported in Koi carp (Udomkusonsri et al., 2007) but lower than the values (80.95 h) reported in Crucian carp at the same dose rate (Shan et al. 2018). The Clearance (Cl/F) in the present study was 0.06 $L.h^{-1}.Kg^{-1}$ which is comparable to the clearance value (0.04 $L.h^{-1}.Kg^{-1}$) reported in Crucian carp (Shan et al., 2018). However, Udomkusonsri et al. (2007) reported a higher clearance of 0.121 $L.h^{-1}.Kg^{-1}$ in Koi carp at the same dose rate. The volume of distribution (V_d/F) after the intramuscular route was 3.75 L/kg, and it was higher than the values (3.1 L/kg) reported in Koi carp (Udomkusonsri et al., 2007) but lower than the values (5.23 L/kg) reported in crucian carp (Shan et al., 2018).

The C_{max} (4.785 µg/mL) observed in the present following bath exposure was higher than the C_{max} values reported in Koi carp (0.86 µg/mL) by Udomkusonsri et al. (2007) and in Crucian carp (0.36 µg/mL) by Shan et al. (2018). In agreement with our results, Zhang et al (2024) reported a C_{max} value of 4.85 h in Northern snake head fish after exposure to higher concentration of 20 mg/L for 6 h. In another study, Lewbart et al. (1997) reported a C_{max} value of 0.17 µg/mL at 2 h following removal from the bath with a lesser dose rate (2.5 mg/L) in red pacu fish. The C_{max} was found to be greater than in previous studies, which indicates better absorption of the drug in the body of the fish.

The T_{max} in the present study was 4 h following bath exposure. However, lower T_{max} values (0.25 h) were reported in Koi carp by Udomkusonsri et al. (2007) following a similar dose of exposure. Lewbart et al. (1997) reported a T_{max} value of 2 h in rep pacu fish following bath exposure of enrofloxacin at the dose of 2.5mg/l. Compared to the previous studies, the T_{max} in the present study was longer, implying a slower rate of absorption of enrofloxacin following bath exposure.

Enrofloxacin exhibited higher AUC values (75.45 $\mu g.h.mL^{-1}$) in rohu following bath exposure than yellow cat fish (22.08 $\mu g.h.mL^{-1}$, Jia et al, 2024), Koi carp (29.1 $\mu g.h.mL^{-1}$, Udomkusonsri et al., 2007) and Crucian carp (14.91 $\mu g.h.mL^{-1}$, Shan et al., 2018) which indicates better availability of the drug in rohu. Urzua et al (2020) reported AUC values of 9.83 and 12. $\mu g.h.mL^{-1}$ in Rainbow trout after bath exposure at 20 mg/L for 2.5 h and 100 mg/L for 0.5 h, respectively and it was lower compared to our results. The MRT of enrofloxacin (27.49 h) in the present study was higher than the value reported (75.3 h) in Koi carp by Udomkusonsri et al. (2007). The Clearance (Cl/F) of enrofloxacin following bath exposure (0.07 $L.h^{-1}.Kg^{-1}$) was lower than that in Koi carp with the same dose rate (0.172 $L.h^{-1}.Kg^{-1}$, Udomkusonsri et al., 2007). The volume of distribution (Vd/F) following bath exposure was lower (2.07 L/kg) compared to Koi carp (10.4 L/kg, Udomkusonsri et al., 2007) at the same dose rate.

Urzua et al – Rainbow trout – Bath immersion 20 ppm for 2.5 h and 100 ppm for 0.5 h – t1/2 – 42.77 and 44.67h; AUC – 9.83 and 12.83.

In the present study, a half-life ($t_{1/2}$) of 21.66 h was observed following bath exposure, which was lower than the values reported in Koi carp (42.1 h, Udomkusonsri et al., 2007) and Crucian carp (62.17 h, Shan et al., 2018) with the same dose rate, i.e., 5 mg/L for 5 hours. However, slower elimination was reported in Northern snakehead fish (90.31 h) by Zhang et al (2024) after bath exposure at 20 mg/L for 6 h and in Rainbow trout (42.77 and 44.67 h) by Urzua et al (2020) following bath exposure at 20 mg/L for 2.5 h and 100 mg/L for 0.5 h, respectively. The

elimination half-life obtained in the present study indicates faster elimination of enrofloxacin in rohu compared to other fishes.

The C_{max} (3.38 µg/mL) observed after oral administration of enrofloxacin is in agreement with the values reported by Shan et al. (2018) in Crucian carp (3.24 µg/mL). In another study, slightly higher C_{max} value of 4.21 µg/mL was reported in Nile tilapia by Corum et al (2022) at the same dose rate. However, a higher C_{max} (14.36 µg/mL) was reported by Udomkusonsri et al. (2007) in Koi carp, whereas Fang et al. (2016) reported a lower C_{max} of 1.86 µg/mL in snakehead fish following oral administration of enrofloxacin at the same dose level. Variable C_{max} values are reported for enrofloxacin in various fishes. C_{max} was reported to be 0.8 µg/mL in red pacu fish (Lewbart et al., 1997) and 1.39 µg/mL in seabass (Intorre et al., 2000) after single oral administration at 5 mg/kg body weight. In largemouth bass, C_{max} was reported to be 10.99 µg/mL after a single oral administration at 20 mg/kg body weight (Shan et al., 2020).

In the present study, the T_{max} of enrofloxacin after the oral route was 8 h. Several reports showed a wide range of Tmax in various fish species. Following a single oral dose of 10 mg/kg body weight, the values recorded in different fish species were 2 h in Nile tilapia (Corum et al, 2022), 3.31 hours in snakehead fish (Fang et al., 2016), 0.68 hours in Crucian carp (Shan et al., 2018), and 0.25 hours in Koi carp (Udomkusonsri et al., 2007). The T_{max} observed in the present study was higher than the reported values, indicating a slower rate of absorption of enrofloxacin in rohu. Similar to our results, Intorre et al. (2000) reported a T_{max} of 8 h in Sea bass after a single oral dose of 5 mg/kg and Shan et al. (2020) reported a T_{max} of 10.99 h in largemouth bass after a single oral dose of 20 mg/kg body weight. However, Lewbart et al. (1997) reported a very high T_{max} value of 36 h in red pacu fish after an oral dose of 5 mg/kg body weight. This variation in the values could be due to the difference in species, age of the fish, etc.

The area under the curve ($AUC_{0-\infty}$) of enrofloxacin in rohu after oral administration was 76.69 µg.h.mL^{-1} and it was comparable to the values reported by Jia et al (2024) in yellow cat fish (88.96 µg.h.mL^{-1}), Udomkusonsri et al. (2007) in Koi carp (156.4 µg.h.mL^{-1}) and by Shan et al. (2018) in Crucian carp (162.72 µg.h.mL^{-1}). However, lower AUC values were reported in Rainbow trout (21.08 µg.h.mL^{-1}) by Urzua et al (2024) and in Snakehead fish (49.98 µg.h.mL^{-1}) by Fang et al. (2016) after an oral dose of 10 mg/kg body weight. In studies conducted with a lesser dose (5 mg/kg body wt.), AUC was reported to be 26.5 µg.h.mL^{-1} in red pacu fish (Lewbart et al., 1997) and 65.93 µg.h.mL^{-1} in sea bass (Intorre et al., 2000). In largemouth bass, AUC was reported to be 1185.73 µg.hr.mL^{-1} after a single oral dose of 20 mg/kg body weight (Shan et al., 2020).

The MRT value of enrofloxacin after the oral route was 40.82 h and it was comparable to the MRT value (43.48 h) reported in sea bass by Intorre et al. (2000) following an oral dose of 5 mg/kg body wt. However, Udomkusonsri et al. (2007) reported lower MRT values of 23 h in Koi carp at the same dose.

Enrofloxacin exhibited a half-life ($t_{1/2}$) of 34.65 h after oral administration in rohu. Similar to the present study results, Fang et al. (2016) reported $t_{1/2}$ of 35.80 h after an oral dose of 10 mg/kg body weight. However, varied $t_{1/2}$ values were reported at the same dose level in various fishes. Udomkusonsri et al. (2007) reported lower $t_{1/2}$ of 16.6 h Koi carp, whereas higher t1/2 values of 56.47 h and 62.17 h were reported in yellow cat fish (Jia et al, 2024) and in crucian carp (Shan et al., 2018), respectively, at the same dose level. Similarly, Shan et al. (2020) reported higher $t_{1/2}$ values of 90.79 h in largemouth bass after a single oral dose of 20 mg/kg body weight.

The clearance of enrofloxacin in rohu (0.13 L.h^{-1}.Kg^{-1}) after oral administration was higher compared to the clearance of enrofloxacin reported in Koi carp (0.064 L.h^{-1}.Kg^{-1}) by Udomkusonsri et al. (2007) and in Crucian carp (0.06 L.h^{-1}.Kg^{-1}) by Shan et al. (2018). However, a higher clearance (0.20 L.h^{-1}.Kg^{-1}) value was reported in snakehead fish after an oral dose of 10 mg/kg body weight. (Fang et al., 2016). In another study with a higher dose rate (20 mg/kg body wt.), a lower clearance value (0.017 L.h^{-1}.Kg^{-1}) was reported in largemouth bass (Shan et al., 2020). In the present study, the volume of distribution (V_d/F) of enrofloxacin was 6.52 L/kg, which was higher than the values reported for Koi carp (1.5 L/kg, Udomkusonsri et al., 2007) and Crucian carp (5.51 L/kg, Shan et al. (2018) at the same dose level. In another similar study, Vd was reported to be 2.21 L/kg in the largemouth bass after a single oral dose of 20 mg/kg body weight (Shan, 2020).

In the present study, higher C_{max} values were obtained in the intramuscular and lower Cmax

values in the oral route, indicating that enrofloxacin absorption was better in the intramuscular compared to other oral and bath exposures. The AUC of enrofloxacin was higher following the intramuscular route than other routes, indicating better absorption than the intramuscular route. However, AUC values were comparable following oral administration and bath exposure. In the present study, Vd ranged from 2.07 L – 6.52 L following different routes of administration, indicating the wide distribution of enrofloxacin in the body tissues of rohu by all routes. The larger Vd of enrofloxacin could be due to its high lipophilic solubility and low (≤8.8%) plasma protein binding (Uney et al., 2021). In the present study, the clearance rate was found to be higher in the oral route compared to other routes. Clearance of enrofloxacin in rohu was comparable following intramuscular and bath exposure. The half-life is very short following bath exposure, which is reflected by shorter MRT, indicating shorter persistence of the drug following bath exposure. MRT was found to be lower following bath exposure compared to the other two routes of administration.

Integration of pharmacokinetic (PK) and pharmacodynamic (PD) parameters is done to assess the clinical efficacy of an antimicrobial agent. The area under the plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}) are the widely used PK parameters, while minimal inhibitory concentration (MIC) is the most commonly used PD parameter. The three PK-PD indices are $AUC_{24\ h}$/MIC, C_{max}/MIC, and T > MIC.

For concentration-dependent antibacterials like enrofloxacin, $AUC_{24\ h}$/MIC and C_{max}/MIC are the best indicators of the clinical efficacy of the drug (Uney et al., 2021). Since MIC studies were not conducted in this study, MIC values reported by other authors were used for PK-PD integration. The MIC_{90} values for fish pathogens like *Aeromonas hydrophila* and *Flavobacterium columnare* were reported to be 0.25 µg/mL (Zhao et al., 2019) and 0.125 µg/mL (Declercq et al., 2013), respectively.

The pharmacodynamics predictors of $AUC_{24\ h}$/MIC ratios ≥ 125 and C_{max}/MIC ratios ≥ 10 have been reported to provide clinical and bacteriological success and prevent resistance emergence (Shan et al., 2022). Considering the MIC_{90} value of 0.30, $AUC_{24\ h}$/MIC and C_{max}/MIC of enrofloxacin were found to be above 125 and 10, respectively, following all three routes of administration and it satisfies the recommended surrogate values, which suggest high efficacy of the drug against common pathogens of fish, *Aeromonas hydrophila* and *Flavobacterium columnare*.

4. CONCLUSION

In conclusion, we found that enrofloxacin achieves sufficient plasma concentration following intramuscular, oral, and bath exposure and can be used for the treatment of bacterial infections of fish with MIC of 0.3 µg/mL.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ANIMAL WELFARE AND ETHICAL APPROVAL

This Project was approved by the research approval committee of the Institute of Fisheries Post Graduate Studies, TNJFU, Vaniyanchavadi, Chennai-603103.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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