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Review Article

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Pharmacokinetics study of Levofloxacin in Goats

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Abstract

Levofloxacine is a third generation Fluoroquinolones with high activity against gram positive and gram-negative bacteria. The present study entitled "Pharmacokinetics study of Levofloxacin after intramuscular administration in Goats ".Total six animals were selected for this particular study and each animal was administered with Levofloxacin @ 5mg /kg body weight intramuscularly 2-3ml venous whole blood were collected from jugular vein into heparinized vacutainer at different time interval.All the blood samples were centrifuged at 3000 rpm for 15 minutes to separate plasma.The plasma sample of Levofloxacin was analyzed by HPLC.

Key words: Levofloxacin, Goats, intramuscular,HPLC,

Introduction: Levofloxacin is a fluoroquinolone antibiotic and is the optical S-(-) isomer of the racemic drug substance ofloxacin, having improved activity against *Streptococcus Pneumonia*. Levofloxacin is a third-generation quinolone with high activity against a broad spectrum of gram-positive penicillin-susceptible and resistant strains of *Streptococcus pneumonia,* gram-negative species *Enterobacter cloacae* and *Proteus mirabilis* and the atypical bacteria *Chlamydophila pneumoniae, Legionella pneumophila* and *Mycoplasma pneumoniae* (Urvesh Patel *et al.*, 2012). Levofloxacin effectively treats upper and lower respiratory tract infections, genitourinary system infections, skin and soft tissue infections, anthrax, endocarditis, meningitis, and travelers' diarrhea (Martinez *et al.*, 2006). It has bactericidal activity at relatively low concentrations and is achieved through reversible binding to DNA gyrase and subsequent inhibition of bacterial DNA replication and transcription (Tripathi,2004). Fluoroquinolones act by a concentration-dependent killing mechanism,

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whereby the optimal effect is attained by administering high doses over a short period of time. This concentration-dependent killing profile is associated with a relatively prolonged post-antibiotic effect (Goudah & Hasabelnaby, 2010). Levofloxacin is widely distributed throughout the body and penetrates well into most body fluids. Its uptake by phagocytic cells makes it suitable for use against intracellular pathogens; Levofloxacin is excreted through urine as an unchanged drug, and less than 5% is metabolized in the liver. The causative agent of mastitis in caprine is mostly staphylococcus species; results have shown that levofloxacin works well in caprine mastitis (Ram *et al.*, 2011).

Materials And Methods

The present study entitled "Pharmacokinetics study of Levofloxacin, Flunixin in goat". The study was carried out at the ILFC, Mumbai Veterinary College, Goregaon. The selected goats were healthy, free from any sign of disease or disease condition. During the entire study period, the goats were kept in a separate enclosure. Fresh concentrate and roughage mixture, and water were provided to the animals. After the experiment was over, the animals were made to join the rest of the herd after completion of experiment. For using goats, the permission was obtained from MVC/IAEC/ 09/2022 and CPCSEA 230/GO/ReBiBt-/S/Re- L/ 2000/ CPCSEA respectively.

Studies in goats

Six clinically healthy goats were selected from the farm. All injections were injected at zero time into the thigh muscles. The dosage was selected on the basis that recommended for using goats. Levofloxacin 5mg per kg body weight in the right gluteal muscle.

Blood sample collection

The blood samples were collected from right jugular vein into heparinized vacutainer at zero (blank sample), 0.166(10 min), 0.33(20min), .5(30min), 1 (60 min), 2(120min), 4(240min),6(360 min), 8 (480min) 10(600 min), 12(720 min), 24(1440 min), 36(2160 min), 48(2880min) hours after treatment. All the blood samples were centrifuged at 10000rpm for 15 minutes to separate plasma. The plasma sample was kept at -70 degree celsius until analysis by HPLC for Levofloxacin and Flunixin assay.

Analysis of samples

1.1 High Performance Liquid Chromatography (HPLC)

1.2 Apparatus

The liquid chromatography(shimadzu) HPLC system consisted of quaternary pump (model DGU-20A5R), Autosampler (Model SIL-20ACHT) and UV Detector (Model CTO – 10 ASVP).

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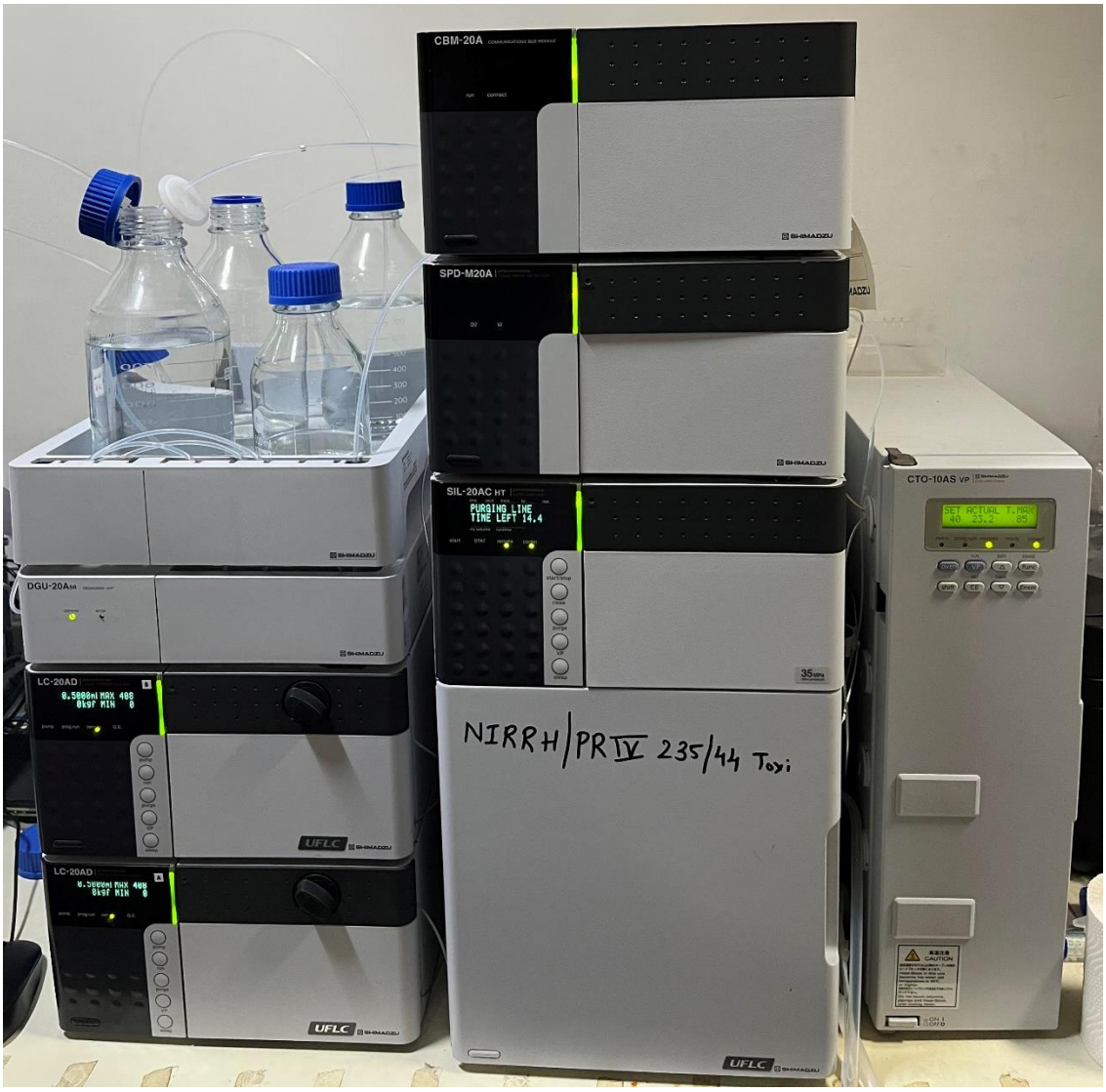

Plate1.High Performance Liquid Chromatography (HPLC)

1.3 Principle of HPLC

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressure of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

2.1 High-Performance Liquid Chromatography (HPLC)

The analysis of Levofloxacin was carried out by using the HPLC method. HPLC analysis of Levofloxacin was modified and carried out as per the method described by Indian Pharmacopeia (2007).

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4.2.1.1 Method validation

4.2.1.1.1 Specificity

4.2.1.1.1.1 Placebo study

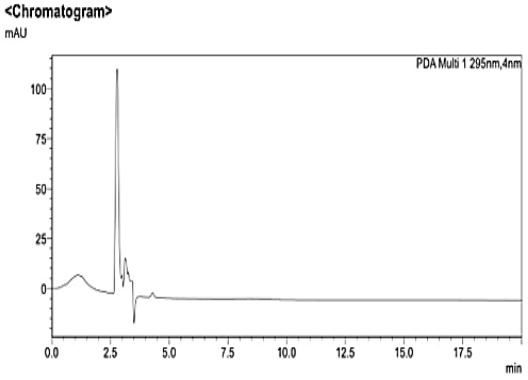

Fig 1. Chromatogram of Placebo mixture (Levofloxacin)

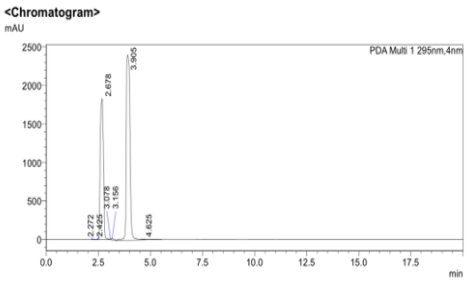

Fig 2. Chromatograph of Levofloxacin (mobile phase)

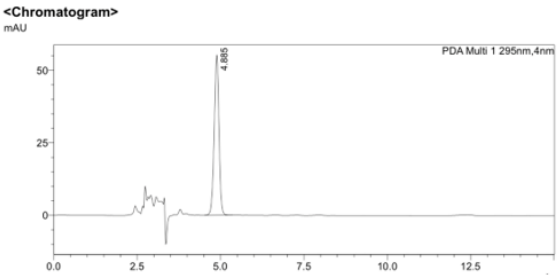

Fig 3. Chromatogram of drug and internal Standard

4.2.1.2. Linearity

4.2.1.2.1 Linearity of Levofloxacin in the mobile phase

The calibration curves of plasma were prepared with different concentrations between 0.01 and 10 ug/ml in the mobile phase. The method was linear in the selected concentration range with a regression coefficient of 0.999 and slope intercepts of 53840.8 and 868.398.

Tab 3. Linearity of Levofloxacin in the mobile phas

Sr. No.	Concentration (ug/ml)	Area
1	10	594881
2	5	302588
3	1.25	94352
4	0.32	31019
5	0.16	16095
6	0.04	15600

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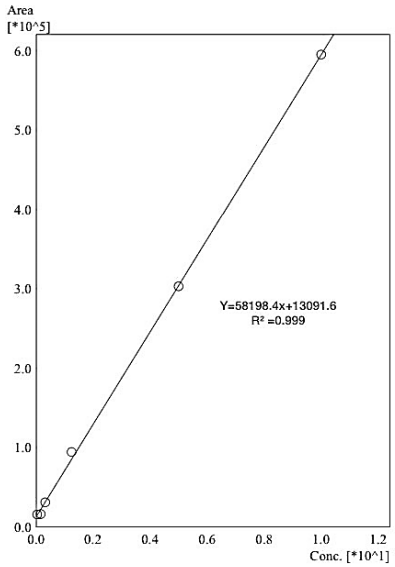

Fig 9. Standard calibration curve of Levofloxacin

4.2.1.3. System Precision

The % RSD of the Area of six replicate injections of standard solution (10ug/ml) was calculated to be 0.148446% which meets the system suitability criterion of % RSD NMT 2%

Tab 4. System precision (RSD)

Injection number	Retention time (min)	Area
1	4.848	801,941
2	4.852	793.053
3	4.857	791,963
4	4.856	792,624
5	4.865	795,461
6	4.867	795,355
Mean	4.857	795,066
%RSD	0.148446	0.46093
STD DEVIATION	.007211	3,664.70

4.2.1.4. Repeatability/ Intra-assay precision

Repeatability is a measure of the precision under the same operating conditions over a short interval of Time, that is, under normal operating conditions of the analytical method with the same equipment. It is also referred to as intra- assay precision. The Table below shows the variation obtained after injecting six ten ug/ml samples from six different weights. The % RSD of Levofloxacin content was 0.460, indicating the precise method.

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Tab. 5 Repeatability Intra-assay precision

Sample	Area
1	801,941
2	793.053
3	791,963
4	792,624
5	795,461
6	795,355
AVG	795,066
%RSD	0.46093
STD DEVIATION	3,664.70

4.2.1.5. Intermediate precision (Ruggedness)

Intermediate precision, expressed within laboratory variations, was evaluated based on days. Data obtained from the inter-day precision assay are given in the Table below.

Tab 6. Intermediate precision

Sample	Area (day 1)	Area (day 2)
1	766404	801941
2	776795	793053
3	774830	791963
4	787642	792624
5	786991	795461
6	776795	795355
Average/mean	778533	795066
%RSD	1.14591	0.46093
Standard deviation	8921.30	3664.70
Pooled data for 12 values		
Mean	786799.5	
Standard deviation	6293	
% RSD	0.80	

4.2.1.6. Robustness

Robustness is the measure of analytical procedure capacity to remain unaffected by minor but deliberate variations in method parameters, which indicates reliability in a normal range.

The flow rate of the mobile phase was 1.0ml/min, while the other mobile phase components were held constant.

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The effect of percent organic strength on the resolution was studied by varying acetonitrile from -5 to +5 (15 to 20) while the other mobile phase components were held constant. The standard solution and sample solution were injected at each changed condition.

4.4.1. Pharmacokinetic parameters of Levofloxacin alone by intramuscular route in goats

After intramuscular administration of Levofloxacin alone, the plasma concentration at different times and a summary of the estimated kinetic parameters in the goat are presented in Table 10. The mean Cmax, Tmax, AUC (0-24hr), CL, Vd, t1/2, and MRT of Levofloxacin was 2.19 ug/ml, 40 min, 37.16 ug/ml*ml, 0.089 mg/ug/ml) min, 125.94 mg/ug/ml, 146.13 min (2.43h), and 258.62 min (4.31 h) after intramuscular administration of Levofloxacin alone at a 5mg/kg body weight dose in goats.

Tab 11. Pharmacokinetic Parameters after intramuscular administration of Levofloxacin alone at a dose rate of 5mg/kg in goats

Parameters	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Mean	SD	SE
Cmax (µg/ml)	1.818	2.028	2.312	2.5	2.218	2.274	2.19	0.217	0.147
Tmax (min)	20	30	20	30	120	20	40	36.05	2.31
AUC (µg/ml*min)	49.64	56.81	85.59	10.70	10.56	9.81	37.16	28.99	4.75
Clearance (mg/µg/ml)mi	0.142	0.105	0.0272	0.112	0.084	0.065	0.089	0.036	0.122
Vd(mg)/µg/ml	39.43	145.39	69.27	129.87	267.94	103.79	125.94	54.85	6.01
T half (min)	192.8.79	96.1.26	175.98	80.01	220.64	111.17	146.13 (2.43 h	72.73	4.88
MRT (min)	257.67	222.80	452.28	105.72	285.85	227.41	258.62 (4.31 h	103.18	6.41

(SD= Standard deviation; SE= Standard error, The Levofloxacin was administered to the six goats at the dose rate of 5mg/kg body weight by the intramuscular route, and the sample were analyzed by HPLC).

After intramuscular administration of Levofloxacin alone, the plasma concentration at different times and a summary of the estimated kinetic parameters in the goat are presented in Table 10. The mean Cmax, Tmax, AUC (0-24hr), CL, Vd, t1/2, and MRT of Levofloxacin was 2.19 ug/ml, 40 min, 838.03 ug/ml*ml, 0.089 mg/ug/ml) min, 125.94 mg/ug/ml, 258.62 min, and 125.94 min after intramuscular administration of Levofloxacin alone at a 5mg/kg body weight dose in goats. In the present study, following intramuscular administration of Levofloxacin, with a dose rate of 5mg/kg body weight, the mean peak plasma concentration of 2.19 ug/ml was achieved at 40 minutes. This

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value is slightly lower than 3.10 ug/ml at 1.64 hours (108.4 min) following intramuscular administration of Levofloxacin at 4mg/kg in sheep (Goudah & Hasanbelnaby, 2010). During this experiment, Cmax was observed at 2.19ug/ml at Cmax 40 minutes; this value of Cmax was lower than 12.25ug/ml as investigated in crossbred cows by Monika and Nitesh 2016. Following intramuscular administration of Levofloxacin Cmax (the peak plasma), Levofloxacin was attained at 40 min in the present study. However, a higher Cmax of 11.25ug/ml at 1 hour in crossbred calves (Monika & Nitesh, 2016), 3.3 ug/ml at 30min in rabbits (Sitovs et al., 2020), 2.95 ug/ml at 1 hour in buffalo calves (Dumka et al., 2007). The differences in Cmax and Tmax might be due to differences in dose. In the present study AUC of Levofloxacin after intramuscular administration was 37.16 ug/ml*min as in rabbits 9.03 mg/ml*h, @ 5mg/kg body weight (Sitovs et al., 2020), 7.32 ug/ml*h in poultry at 10mg/kg body weight (Bisht et al., 2018), 8.81 ug/ml*h in buffalo calves at 3mg/kg and 66.316 ug/ml*min in crossbred cows at 4mg/kg body weight. In the present study, after intramuscular administration of Levofloxacin at 5mg/kg body weight in six goats, the mean of total body clearance (CL) was 0.089 mg/ml/min, was as in poultry 1401.3mg/ml/min (Bisht et al., 2018), buffalo calves 343.2mg/g/h) (Dumka et al., 2007) after intramuscular administration of Levofloxacin. In the present experiment, the apparent Vd of Levofloxacin was 185.09mg/ug/ml, whereas in buffalo calves, 1.06L/kg (Dumka et al., 2007), in crossbred cows, 0.306ml/kg (Monika & Nitesh, 2016) and poultry 5925ml/kg. Elimination t1/2 and MRT in the goat were 146.13 (2.43 h) and 258.62 min (4.3h), respectively. Almost similar values of t1/2 and MRT were observed in crossed-bred cows (Monika & Nitesh, 2016), buffalo calves (Dumka et al., 2020), and Poultry (Bisht et al., 2018) after intramuscular administration of 5mg/kg Levofloxacin. These values may differ due to dose, age, species, and environmental differences.

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