PHARMACOKINETIC AND BIOAVAILABILITY STUDIES OF ENROFLOXACIN AND CIPROFLOXACIN IN GOATS

Thesis

SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE
OF

Doctor of Philosophy

IN

VETERINARY PHARMACOLOGY

BY

S. Ramesh

Roll No 507

TO
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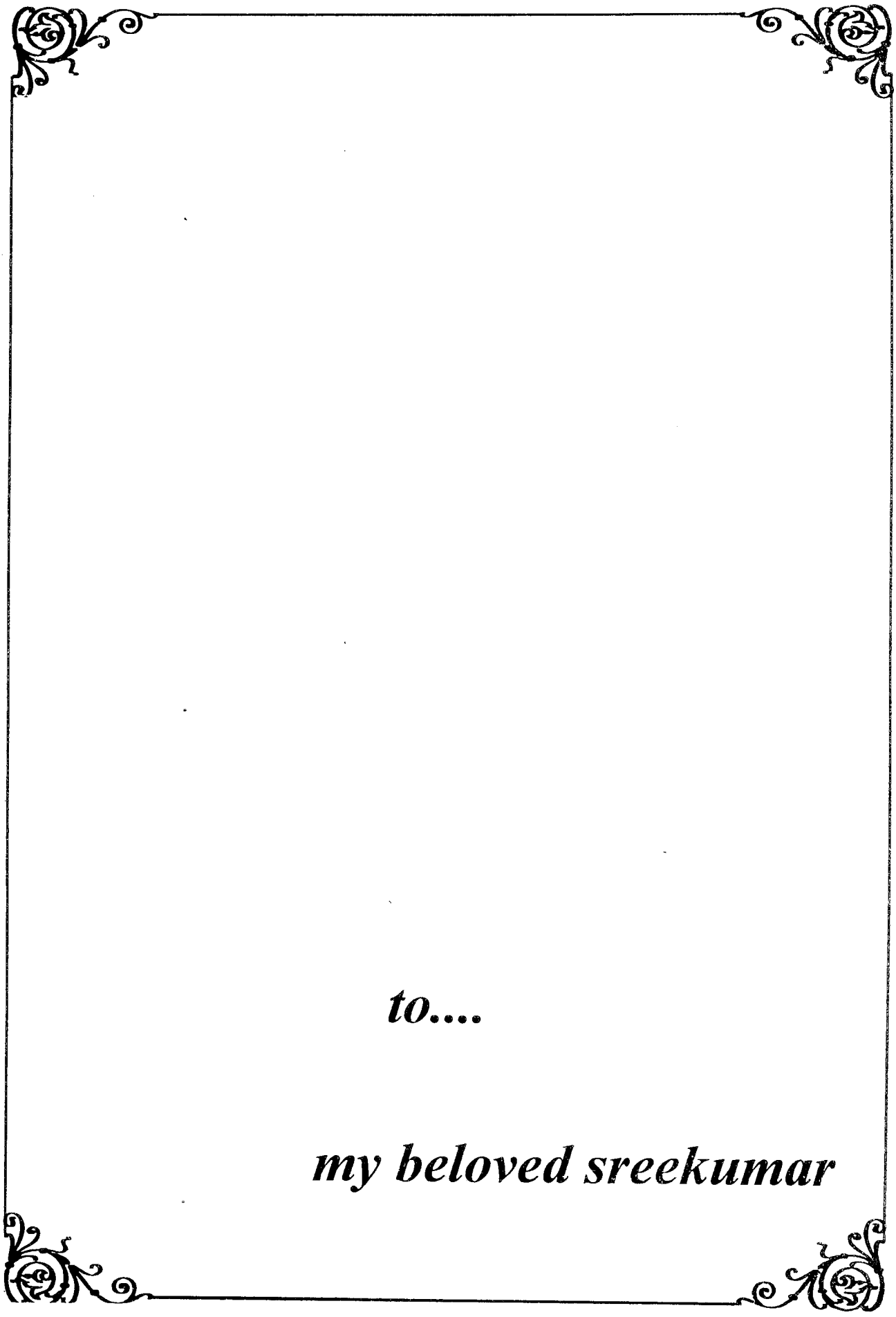

to....

my beloved sreekumar

Dr. J.K. Malik
M.V.Sc.,Ph.D.
Head

Division of Pharmacology & Toxicology
Indian Veterinary Research Institute,
Izatnagar (U.P.) - 243 122

Dated : 17.1.2001

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*Certified that the research work embodied in this thesis entitled, "**Pharmacokinetic and bioavailability studies of enrofloxacin and ciprofloxacin in goats**" submitted by **Dr. S. Ramesh**, Roll No. 507 for the award of the Degree of **Doctor of Philosophy** in **Veterinary Pharmacology** of Indian Veterinary Research Institute, is the original work carried out by the candidate himself under my supervision and guidance.*

*It is further certified that **Dr. S. Ramesh** Roll No. 507, has worked for more than 30 months in this institute and has put in more than 300 days attendance under me from the date of registration for the degree of Doctor of Philosophy at this Deemed University as required under the relevant ordinance.*

17.1.2001
(J.K. Malik)
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17.1.2001
(J.K. Malik)
Signature of Chairman
Advisory Committee
Date : the 17th Jan. 2001

MEMBERS OF STUDENT'S ADVISORY COMMITTEE

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Timely help, albeit tiny, is ever
greater than the universe

- Thiruvalluvar (Tamil)

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Abbreviations

A	:	Zero-time intercept of distribution phase
A'	:	Zero-time intercept of absorption phase
α	:	Distribution rate constant
AUC	:	Area under the plasma concentration-time curve
AUMC	:	Area under the moment curve
B	:	Zero-time intercept of elimination phase
β	:	Elimination rate constant
CIP	:	Ciprofloxacin
C_{max}	:	Maximum (peak) plasma concentration
$C_{max\,(obs)}$	:	Maximum (peak) observed plasma concentration
Cl_B	:	Total body clearance
C_p	:	Plasma concentration
C_p^0	:	Theoretical concentration of drug in plasma at zero-time
e	:	Base of normal logarithm
E. coli	:	*Escherichia coli*
ENR	:	Enrofloxacin
F	:	Bioavailability/fraction of drug absorbed after non-vascular administration
F_c	:	Fraction of drug in central compartment
h	:	Hour
i.m.	:	Intramuscular
i.v.	:	Intravenous
k_{12}	:	Rate of transfer of drug from central to peripheral compartment
k_{21}	:	Rate constant of transfer of drug from peripheral to central compartment
k_{el}	:	Elimination rate constant of drug from central compartment
k_a	:	Absorption rate constant
k_f	:	Metabolite formation rate constant
kg	:	Kilogram(s)
L	:	Litre(s)
MAT	:	Mean absorption time
µg	:	Microgram(s)
mg	:	Milligram(s)

MIC	:	Minimum inhibitory concentration
min	:	Minute(s)
ml	:	Millilitre(s)
MR	:	Metabolite ratio
MRT	:	Mean residence time
$MRT_{i.v.}$	:	Mean residence time of drug after intravenous administration
$MRT_{n.v.}$	:	Mean residence time of drug after nonvascular administration
po	:	*per os* (oral)
s.c.	:	Subcutaneous
S.E.	:	Standard error
t	:	time
$t_{1/2\,\alpha}$	:	Distribution half-life
$t_{1/2\,\beta}$	:	Elimination half-life
$t_{1/2\,ka}$	:	Absorption half-life
$t_{1/2\,kf}$	:	Metabolite formation half-life
T/P ratio	:	Tissue/Plasma ratio
$t_{max\,(obs)}$	:	Time of maximum observed concentration in plasma
V_c	:	Volume of central compartment
$V_{d(area)}$	:	Apparent volume of distribution
$V_{d(SS)}$	:	Volume of distribution at steady-state
V_p	:	Volume of peripheral compartment

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INTRODUCTION

Fluoroquinolones are a new class of antimicrobials that are being extensively investigated currently for use in both human and veterinary medicine. They are bactericidal at low concentrations, possess broad spectrum of activity, well absorbed and widely distributed in body tissues with low host toxicity (Brown, 1996). Two of the most commonly used fluoroquinolones are enrofloxacin and ciprofloxacin. The former drug is approved for veterinary use and its chemical structure is similar to that of ciprofloxacin, differing only in the addition of an ethyl group to the piperazinyl ring. Enrofloxacin is metabolised in the body and the main metabolite is ciprofloxacin, which is itself licensed for use in human and medicine.

Pharmacokinetic studies offer highly relevant information on the time-course of the drugs and their metabolites and facilitiate the computation of optimal dosage regimens of drugs to maintain their therapeutic concentration at the biophase

(Gibaldi and Perrier, 1982; Notari, 1987). The pharmacokinetic behaviour of fluoroquinolones is being investigated with great interest in various animal species. The pharmacokinetic properties of enrofloxacin have been reported in cattle (Kaartinen *et al.*, 1995), horses (Langston *et al.*, 1996; Kaartinen *et al.*, 1997a), pigs (Zeng *et al.*, 1996; Anadon *et al.*, 1999), dogs (Walker *et al.*, 1992; Kung *et al.*, 1993), chicken (Anadon *et al.*, 1995; Garcia *et al.*, 1999) and in a variety of wild animal species (Intorre *et al.*, 1997; Lewbart *et al.*, 1997). Similarly, the pharmacokinetics of ciprofloxacin has been investigated in calves and pigs (Nouws *et al.*, 1988), sheep (Munoz *et al.*, 1996), dogs (Abadia *et al.*, 1994), ponies (Dowling *et al.*, 1995) and broiler chicken (Atta and Sharif, 1997). However, the detailed pharmacokinetic studies of enrofloxacin and ciprofloxacin are lacking in goats, which is an economically important livestock species in India. Marked species variations in drug disposition make it difficult to extrapolate the pharmacokinetic data established in other species to goats. Furthermore, pharmacokinetic studies are relevant in the species and the environment in which the drug is to be used clinically. It is, therefore, of utmost importance to investigate the pharmacokinetics and bioavailability of enrofloxacin and ciprofloxacin in goats.

Although fluoroquinolones possess an excellent oral bioavailability in monogastric animals, their bioavailability by oral route is relatively poor in ruminants (Jenkins, 1990). This necessitates the use of parenteral routes of administration for this class of drugs. There is also an increasing demand from beef producers for drug formulations suitable for subcutaneous administration (Clarke *et al.*, 1999) to reduce the damage to muscle tissues and subsequent loss of marketable beef. The superiority of subcutaneous administration has already been documented for various antimicrobial drugs including sulphamethoxypyridazine in goats (Garg and Uppal, 1997), cephalexin in calves (Garg *et al.*, 1996), gentamicin in cats (Jernigan *et al.*, 1988a), enrofloxacin in calves (Martinez-Larranaga *et al.*, 1997) and cattle (Stegemann *et al.*, 1997). The advantages of the

subcutaneous route are ease of administration, good bioavailability and maintenance of therapeutic drug concentration for longer duration (Jernigan *et al.*, 1988b). No pharmacokinetic data of enrofloxacin and ciprofloxacin seem to be available after their subcutaneous administration in goats. Thus, in the present study, it was proposed to investigate the pharmacokinetics and bioavailability of these two fluoroquinolone antimicrobials in goats following their subcutaneous administration.

Disease states are known to alter the pharmacokinetics of drugs. The pharmacokinetic data normally generated in healthy animals may not be appropriate for use in diseased animals. Endotoxaemia is a condition arising out of release of endotoxin from bacterial pathogens in the body, causing fever and related clinical symptoms that is known as acute phase response. Since antibacterials, including fluoroquinolones are often used in these clinical circumstances, it is essential to understand the influence of endotoxaemia on the disposition of these drugs. Accordingly, the effect of endotoxin-induced fever has been investigated on the pharmacokinetics disposition of chloramphenicol in goats (Kume and Garg, 1986), gentamicin in horses (Wilson *et al.*, 1983), ewes (Wilson *et al.*, 1984) and cats (Jernigan *et al.*, 1988a), sulphonamides in lambs (van Miert *et al.*, 1976) and goats (Nouws *et al.*, 1986) norfloxacin in goats (Jha *et al.*, 1996) and enrofloxacin in cross-bred calves (Ahangar and Srivastava, 2000). Little is known about the influence of febrile state on the disposition of enrofloxacin and its metabolite in goats. Since fever is one of the most important cardinal manifestations in various infectious diseases, where enrofloxacin is to be used, the effect of endotoxin-induced fever on the pharmacokinetics of ENR was studied in goats after subcutaneous administration.

Enhanced bioavailability of drugs can help in lowering the drug dosage and increasing their dosing interval. Piperine, an active ingredient of long pepper and black pepper has been reported to enhance the bioavailability of co-administered

black pepper has been reported to enhance the bioavailability of co-administered drugs. Piperine increased the bioavailability of rifampicin (Zutshi *et al.*, 1985) and oxyphenbutazone (Majumdar *et al.*, 1999). However, little is known about the effect of piperine on the bioavailability of enrofloxacin in domestic animals. In the present study, the effect of piperine treatment was investigated on the pharmacokinetics and bioavailability of enrofloxacin in goats.

Probenecid is another drug that is already in use to prolong the plasma concentrations of antimicrobial agents. It acts by inhibiting the transport of organic acids across epithelial barriers (Insel, 1996), prolongs the biological half- life, and alters the distribution of drugs that are mainly secreted by renal tubules (Weling *et al.*, 1985). Since both enrofloxacin and ciprofloxacin are excreted via kidney with the possibility of renal tubular secretion, their elimination may be blocked by probenecid. Accordingly in the present study, the effect of subcutaneous administration of probenecid was investigated on the disposition kinetics of enrofloxacin and ciprofloxacin in goats.

The present study was undertaken in goats with the following objectives :

i) ***To determine the plasma concentrations and pharmacokinetics of enrofloxacin and ciprofloxacin in goats following intravenous and subcutaneous administration.***

ii) ***To study the effect of febrile state on the pharmacokinetics of enrofloxacin.***

iii) ***To study the effect of piperine on the pharmacokinetics and bioavailability of enrofloxacin.***

iv) ***To study the effect of probenecid on the pharmacokinetics and bioavailability of enrofloxacin and ciprofloxacin.***

REVIEW OF LITERATURE

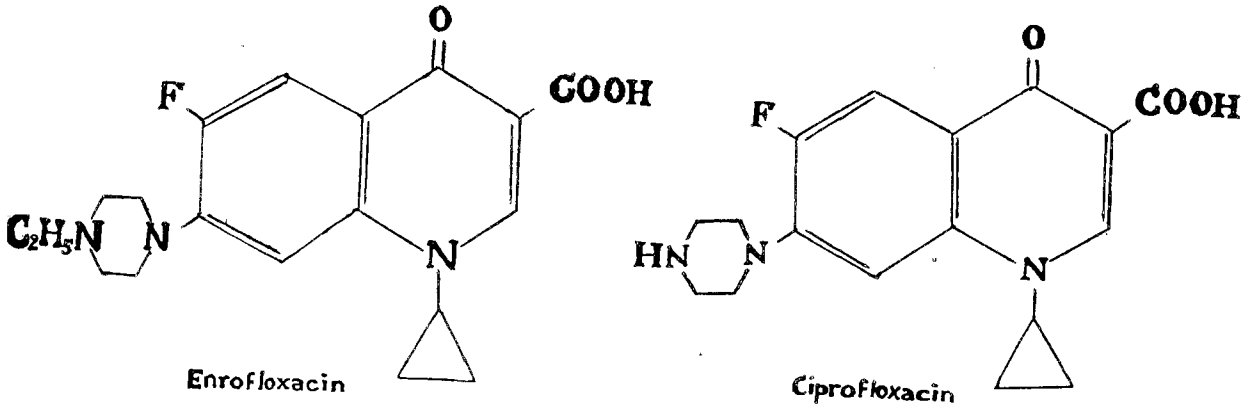
Enrofloxacin and ciprofloxacin are flourinated quinolone carboxyl e acid derivatives. Ciprofloxacin is the most widely used fluoroquinolone in human medicine. Enrofloxacin was developed exclusively for veterinary use. Enrofloxacin is approved in the USA for use in dogs and in Europe for dogs, cats, swine, cattle and poultry. Enrofloxacin is metabolised to ciprofloxacin in the body. Both drugs are characterised by the following properties : wide antibacterial spectrum, good absorption after parenteral administration, high bioavailability and good tissue penetration leading to high concentration of the drug is tissues (Scheer, 1987; Anadon *et al.*, 1995). Both drugs are eliminated predominantly by renal route by glomerular filtration and tubular secretion (Hooper and Wolfson, 1991).

2.1 Physicochemical properties of Enrofloxacin and Ciprofloxacin

Enrofloxacin has a molecular weight of 359.4 and has the following molecular formula $C_{19} H_{22} F N_3 O_3$. It is a pale yellow crystalline powder and is sparingly soluble

in water. It contains both acidic and basic groups and thus behaves as a zwitterion. Aqueous solutions are very stable. The activity of enrofloxacin declines in acidic environment.

The molecular weight of ciprofloxacin is 331.3 and is usually available as hydrochloride salt. It is a light yellow powder, A 2.5% solution has a pH 3-4.5. Ciprofloxacin infusion have a pH of 3.9-4.5 and is incompatible with compounds which are unstable at this pH range.



2.2 Mechanism of action

Enrofloxacin and ciprofloxacin, like other fluoroquinolones, are primarily bactericidal agents. They act by inhibition of DNA gyrase, an enzyme responsible for controlling the supercoiling of bacterial DNA (Neuman, 1988; Hooper and Wolfson, 1991). Bacteria possess a type II topoisomerase (DNA gyrase). This enzyme is responsible for folding and coiling the 1.0-1.3 cm length of circular bacterial DNA, so that it gets compacted into the bacteria, which is several thousand times smaller. The DNA gyrase catalyses alignment of DNA into a relaxed form

that is less susceptible to fragmentation and increases the efficiency of replication during strand replication (Fernandez, 1988). This is done by coiling the DNA around RNA in a series of loops. Each loop or domain is then negatively supercoiled by introducing 'nicks' in both strands of DNA, passing that broken strand behind double strand and then resealing the double nick. The fluoroquinolones inhibit the resealing of the double nick causing degradation of chromosomal DNA. Mammals, too, possess an enzyme that is similar to DNA gyrase but it dose not supercoil the DNA and is not affected by fluoroquinolones (Brown, 1996).

2.3 Antimicrobial spectrum

Fluoroquinolones, in general, are broad spectrum agents. Both enrofloxacin and ciprofloxacin are active against gram negative bacteria, gram positive bacteria and including mycoplasma, chlamydia sp. and ureaplasma. Pathogenic bacteria of clinical importance such as *E. coli*, *Salmonella*, *Klebsiella*, *Yersinia*, *Haemophilus*, *Pasteurella*, *Actinobacillus* and *Moraxella* are highly sensitive with MIC values ranging from 0.008 to 0.06 $\mu g.ml^{-1}$. Bacterial species such as *Serratia*, *Proteus*, *Citreobacter*, *Campylobacter*, *Brucella*, *Bordetella*, *Vibrio*, *Staphylococcus*, *Erysipielothrix*, *Bacillus* and mycoplasmas are moderately sensitive with MIC values of 0.125 to 0.5 $\mu g.ml^{-1}$. Because of structural similarity, enrofloxacin and ciprofloxacin have similar antibacterial spectrum.

Fluoroquinolones are more active in alkaline environment (pH >7.4) against gram negative bacteria and activity against gram positive bacteria is not affected by pH. Susceptibility is not affected by inoculum size, but activity is reduced by divalent cations (Brown, 1996).

2.4 Pharmacokinetics

Pharmacokinetics is the study of time course of drug concentration in the body, which depends upon physiological processes such as absorption, distribution

and elimination. The pharmacokinetic analysis of drugs in a species provides an important tool in the optimisation of dosage regimen of the drug in the species studied.

The pharmacokinetic studies are based on mathematical modelling which are used to describe the changes in concentration over a period of time. There are essentially two approaches in this modelling i) the classical compartmental approach and (ii) the non-compartmental approach.

2.4.1 Compartmental analysis :

In this the body is conceived to be consisting of distribution compartment, one or many, into which drug enters or leaves dictated by rate constants. These compartments are mathematical entities, and have no physiological or anatomical counterparts. The pharmacokinetics of a drug can thus be described by one-compartment or multi-compartment open models.

A one-compartment open model deems the body to be a single homogenous unit. Hence, any change in blood drug concentrations reflects the quantitative changes in tissue concentration. In this model, if the plasma concentration time profile is plotted on a semi-logarithmic scale, a straight line is obtained and the equation for drug decline is as follows :

$$Cp = Be^{-\beta t}$$

where Cp is the plasma concentration of drug, at time t B is the Y-intercept of the regression line, 'ß' is the overall elimination rate constant, 't' is the time elapsed and 'e' is the base of natural logarithm. This model has been found to adequately describe the kinetics of many drugs including chloramphenicol (Varma, 1978), erythromycin (Burrows *et al.*, 1989) and netobimin in calves (Lanusse *et al.*, 1990), quinidine (Neff *et al.*, 1972) and amphetamine in domestic animals (Baggot and Davis, 1973) and norfloxacin in chicken, goose and turkey (Laczay *et*

al., 1998).

The two-compartment open model, assumes the existence of an instantaneous central compartment consisting of the blood and highly perfused organs such as liver, kidney, lungs etc. and a peripheral compartment consisting of less perfused tissues such as muscle, skin, rumen etc. A basic assumption associated with this model is that the elimination takes place exclusively from the central compartment. The equation for describing this model is,

$$Cp = Ae^{-\alpha t} + Be^{-\beta t},$$

where A and B are zero time intercepts of the initial and terminal phases of the concentration-time curve with dimensions of concentration ($\mu g\ ml^{-1}$), α and β are the distribution and elimination rate constants. The constant α and the Y intercept of the distribution phase 'A' are determined by residual analysis.

The constants A, B, α and β are used to calculate many derived parameters such as $t_{1/2\beta}$ elimination half-life, distribution half-life, area under the plasma concentration time curve, clearance, volume of distribution, microrate contants and the multiple dosage regimen. Most of the therapeutic agents used in humans and animals can be satisfactorily described by this two-compartment open model.

Some of the drugs such as, diazepam in man (Kaplan *et al.*, 1973), oxytetracycline in dogs (Baggot *et al.*, 1977) and indomethacin in sheep (Vinagree *et al.*, 1998) are known to follow a three-compartment open model, which is mathematically expressed as

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} + Pe^{-\pi t}$$

The most important pharmacokinetic parameters that ultimately characterise the behaviour of a drug are the $t_{1/12\beta}$, Cl_B and V_d. Gibaldi and Wintraub (1971) defined the half-life as a measure of the rate of drug elimination the time required to reduce

the drug concentration of plasma or serum to its half during the elimination phase. The half-life is inversely proportional to the overall elimination rate constant. This parameter is very important in determining the withdrawal times of drugs and chemicals in food animals.

Apparent volume of distribution ($V_{d\,area}$) is a hypothetical volume of body fluids that could be required to dissolve the total amount of drug at the same concentration as found in blood. This parameter is most helpful for computation of dosage regimens.

Clearance is another important parameter, defined as the amount of body fluid from which the drug is removed per unit time. Clearance is useful in adjustment and maintenance of optimal dosage once the treatment has been initiated.

2.4.2 Non-compartmental analysis :

This method of analysis does not require the assumption of a specific compartmental model for either drug or metabolite. These can be applied to any compartmental model provided the linear pharmacokinetics is ascertained. Of late, there has a been a distinct shift from the curve fitting elaboration of compartmental method towards non-compartmental method of analysis (Gibaldi and Perrier, 1982). While compartmental model holds good satisfactorily explaining the drug behaviour with good curve-fitting experimental data, non-compartmental approach presents a straight forward simpler approach precluding the possible misinterpretation of data due to misspecification (Martinez, 1998).

The basis of non-compartmental analysis is the statistical moments theory (Yamaoka *et al.*, 1978). In pharmacokinetics, the three moments are described by

$$AUC = \int_0^\infty C\,dt \qquad \text{(Zero moment)}$$

$$MRT = \frac{\int_0^\infty tCdt}{\int_0^\infty Cdt} \qquad \text{(First moment)}$$

$$VRT = \frac{\int_0^\infty t^2Cdt}{\int_0^\infty Cdt} \qquad \text{(Second moment)}$$

where AUC is the area under the plasma concentration-time curve, MRT is the mean residence time and VRT is the variance of residence time. The moments defined above are usually calculated by the numerical integration using the trapezoidal rule. While AUC and MRT are invariably reported, the third moment is rarely used.

In non-compartmental analysis, AUC is the basic parameter which serves as the basis for comparison across dosage regimens, formulation, etc., for relating to a pharmacodynamic variable or to identify factors their may affect pharmacokinetics such as disease, food, gender, age, breed, physiological status etc.

Mean residence time (MRT) is an indicator of temporal characteristics of a dose and remains contant, regardless of dose, in the absence of saturable processes MRT, as a parameter helps deduce the mean absorption time, mean dissolution time *etc*. Other important parameters such as clearance and volume of distribution can be derived from AUC/MRT which can ultimately give a complete description of a drug's pharmacokinetics.

2.5 Pharmacokinetics of Enrofloxacin

Enrofloxacin is the most widely used fluoroquinolone in veterinary practice and hence widely reported. Pharmacokinetics of enr ofloxacin has been characterised in a variety of species including wild animals and aquatic species.

Table 1. Disposition kinetics of enrofloxacin in domestic animals and poultry

Species	Dose ($mg.kg^{-1}$)	Route	Pharmacokinetic Parameters				Reference
			$t_{1/2\beta}$ (h)	AUC ($\mu g.h.ml^{-1}$)	Cl_B ($ml.h^{-1}.kg^{-1}$)	$V_{d\ area}$ ($L.kg^{-1}$)	
Calves	2.5	i.v.	4.87		39.0	–	Kaartinen *et al.* (1997)
Chicken	10	i.v.	10.29	34.51	17.4	4.31	Anadon *et al.* (1995)
Dairy cows	5	i.v.	65.4[a]	7.4	1260	2.1[b]	Malbe *et al.* (1996)
Lactating cows	5	i.v.	1.68	7.42	–	–	Kaartinen *et al.* (1995)
		s.c.	5.55	9.62	–	–	
Ilamas	5	i.v.	3.38	6.95	700	3.46[b]	Christensen *et al.* (1996)
Mares	7.5	i.v.	5.33	21.03	370	2.93	Haines *et al.* (2000)
Foals	5	i.v.	17.1	48.54	103	2.49	Bermingham *et al.* (2000)
Pigs	2.5	i.v.	9.64	25.69	100	1.41	Anadon *et al.* (1999)
Buffalo bulls	5	i.m.	1.97	21.5	–	0.61[c]	Verma *et al.* (1999)
Rabbits	5	i.v.	2.5	8.6	606	2.12	Broome *et al.* (1991)
		s.c.	2.41	5.43	–	–	
Sheep	2.5	i.v.	3.73	5.47	550	3.02[b]	Mengozzi *et al.* (1996)
		i.m.	3.65	4.58	620	3.03[b]	
Goats	5	i.m.	1.39	7.82	803	1.52	Rao (1999)

[a] = min; [b] = $V_{d\ SS}$; [c] = $V_{d\ (\beta)}$

Since oral bioavailability of enrofloxacin is greatly reduced in ruminants (Jenkins, 1990), it is administered by parenteral routes, mainly intramuscular and subcutaneous routes (Prescott and Baggot, 1993). Absorption of enrofloxacin after the parenteral administration is fast and it is eliminated primarily via kidneys (Van Cutsem *et al.*, 1990).

2.5.1 Horses

Langston *et al.* (1996) investigated the disposition of enrofloxacin after a single oral dose of 5 $mg.kg^{-1}$ in horses. The mean biological half-life was 7.75 h and the mean AUC was 18.939±14.410 $\mu g.h.ml^{-1}$. The maximum concentration (C_{max}) achieved was 1.853±0.859 $\mu g.ml^{-1}$, at 0.92±0.59 h (t_{max}). Kaartinen *et al.* (1997a) studied the pharmacokinetics of enrofloxacin after single i.v. or i.m. administration at the same dosage. Elimination half-life was considerably longer (9.9 h) after i.m. administration than i.v. injection (4.4 h). Enrofloxacin was rapidly metabolized to ciprofloxacin reaching 20-35% of that of the parent drug.

In mares, after intravenous administration of enrofloxacin (7.5 $mg.kg^{-1}$), the $t_{1/2\beta}$ was 5.33 h with a AUC of 21.03 $\mu g.h.ml^{-1}$. After intragastric administration, the C_{max} was 0.94±0.97 $\mu g.ml^{-1}$. The bioavailability of ENR after intragastric administration of a poultry formulation (32.3 mg/ml) was 78.29% (Haines *et al.*, 2000). However, in foals, Bermingham *et al.* (2000) reported a very long $t_{1/2\beta}$ of 17.10 h after i.v. administration of ENR (5 $mg.kg^{-1}$). After oral administration of ENR (10 $mg.kg^{-1}$), the C_{max} was 2.12 $\mu g.ml^{-1}$ and the bioavailability was 42.0%.

2.5.2 Dogs

Walker *et al.* (1992) reported that the elimination half-life of enrofloxacin was increased from 3.39 h for the 2.75 mg/kg dose to 4.94 h for the 11 mg/kg dose. After i.v. or p.o. administration at 5 $mg.kg^{-1}$, mean elimination half-life of enrofloxacin was 2.4 h, clearance was 27.1 $ml.min^{-1}$ and mean V_{ss} was 7.0 $L.kg^{-1}$ (Kung *et al.*, 1993).

2.5.3 *Pigs*

In both fasted and fed pigs, therapeutically active concentrations of enrofloxacin were maintained upto 24 h after oral administration (10 $mg.kg^{-1}$). The oral bioavailability was 83±13% in fed and 101±32% in fasted pigs (Nielsen and Hansen, 1997). After i.m. administration at 2.5 $mg.kg^{-1}$, enrofloxacin was absorbed rapidly to reach a peak concentration of 0.75 $\mu g.ml^{-1}$ at 0.9 h. The elimination half-life (5.5 h) was similar to that after i.v. injection. The systemic bioavailability was close to 100% (Pijpers *et al.*, 1997).

2.5.4 *Chicken*

After single i.v. and oral dose of 10 $mg.kg^{-1}$, the elimination half-lives were 10.29 ± 0.45 and 14.23 ± 0.46 h, respectively. Enrofloxacin was slowly absorbed with a C_{max} of 2.44 ± 0.01 $\mu g.ml^{-1}$ at 1.64 ± 0.04 h. Oral bioavailability was 64 ± 0.2%. In the chicken, enrofloxacin residues persisted in liver and ciprofloxacin (metabolite) persisted in muscle, liver and kidney upto day 12 post-treatment (Anadon *et al.*, 1995).

Garcia *et al.* (1999) compared the pharmacokinetics of enrofloxacin and ciprofloxacin in chicken after an i.v. administration of both the drugs at a dose of 5 $mg.kg^{-1}$and reported significant differences in the phamacokinetics of enrofloxacin and ciprofloxacin.The $t_{1/2\beta}$ of ENR was reported to be 6.99 ± 0.48 h while that of CIP was 3.11 h.The AUC of ENR (26.76 $\mu g.h.ml^{-1}$) was four-fold higher than that of CIP (5.67 $\mu g.h.ml^{-1}$). CIP was characterised by high Cl_B and V_{dss} as compared to ENR. ENR was converted to CIP to the extent of 10%. These workers suggested the utility of CIP with lesser residual problems than ENR.

2.5.5 *Cattle and buffaloes*

Kaartinen *et al.* (1995) studied the pharmacokinetics of enrofloxacin in lactating cows. After single i.v., i.m. and s.c. administration of enrofloxacin at 5

mg.kg^{-1}, the values of $t_{1/2\beta}$ of antimicrobial activity in serum were 1.7, 5.9 and 5.6 h, respectively with about 36-45% of serum protein binding. The peak concentration (C_{max}) achieved after s.c. administration (0.98 ± 0.20 μg.ml^{-1}) was higher than that after i.m. injection (0.73 ± 0.12 μg/ml). The bioavailability was also higher after s.c. administration (137 ± 31%) than following i.m. administration (82 ± 14%). Ciprofloxacin, rather than enrofloxacin, was trapped more in milk and was responsible for the antimicrobial activity in milk.

There was no significant difference in elimination half-life of the drug between new-born and one-week old calves after i.v. admini stration of enrofloxacin (2.5 mg.kg^{-1}). However, Vd_{SS} and clearance (1.8 L.kg^{-1} and 0.19 L.kg^{-1}.h) were significantly reduced in new-born calves compared to one-week old calves (2.3 L.kg^{-1} and 0.39 L.kg^{-1}.h, respectively). Metabolism to ciprofloxacin was slower in new-born calves (Kaartinen *et al.*, 1997b).

In beef cattle, following subcutaneous administration of enrofloxacin at 7.5 mg.kg^{-1}, the observed C_{max} was 0.83 ± 0.56 μg.ml^{-1} by HPLC method; and 1.71 ± 0.93 μg.ml^{-1} by bioassay. The bioavailability was 110 ± 21.1%. the volume of distribution at steady state was 1.46 ± 0.59 L.kg^{-1} (Stegemann *et al.*, 1997).

In calves too, after s.c. administration of enrofloxacin at 2.5 mg.kg^{-1}, the bioavailability was 96.0 ± 1.0%, indicating almost complete absorption. The elimination half-life was also longer by s.c. (19.08 ± 0.72 h) than after i.v. (16.31 ± 0.77 h) administration (Martinez-Larranaga *et al.*, 1997).

Pharmacokinetic studies of enrofloxacin have also been reported in buffaloes following s.c. and i.v. injections at a dose of 2.5 mg.kg^{-1} body weight (Amorena *et al.*, 1992). After i.v. administration, the initial concentration was 1.75 ± 0.35 μg.ml^{-1}. After s.c. administration, the maximum concentration of 0.210 ± 0.037 μg.ml^{-1} was obtained at 70 min. The half-life of the drug was similar by both routes.

In buffalo bulls, the disposition kinetics and dosage regimen of enrofloxacin were investigated after i.m. administration at a dose of 5 $mg.kg^{-1}$. The $t_{1/2\beta}$ was 1.97 ± 0.23 h. The $V_{d\,area}$ was 0.61 ± 0.13 $L.kg^{-1}$ and the Cl_B was 210.2 ± 18.6 $ml.h^{-1}.kg^{-1}$ (Verma *et al.*, 1999).

2.5.6 Sheep

Disposition kinetics of enrofloxacin in sheep was investigated after i.m. and i.v. administration (2.5 $mg.kg^{-1}$). The drug was detected in serum upto 4 and 8 h after i.v. and i.m. injections, respectively (Pugliese *et al.*, 1991).

The bioavailability of enrofloxacin was 85% after i.m. injection at 2.5 $mg.kg^{-1}$ (Mengozzi *et al.*, 1996). Thirty-five and 55 per cent of the parent drug was converted to ciprofloxacin after i.v. and i.m. administration, respectively. The large volume of distribution indicated wide distribution of the drug in the body. Following oral administration, elimination half-life was longer than both after i.m. and i.v. injections. The oral bioavailability was 60.6% (Pozzin *et al.*, 1997).

2.6. Pharmacokinetics of Ciprofloxacin

Ciprofloxacin is one of the most widely used quinolones in humans and in veterinary medicine. Nouws *et al.* (1988) administered ciprofloxacin at a dose of~3 $mg.kg^{-1}$ by both i.v. and oral routes in pre-ruminant calves and pigs. Ciprofloxacin was rapidly absorbed and well distributed with a short elimination half-life of 2.5 h in both species. The t_{max} was 2-3 h. The oral bioavailability was 53% in calves and 37.13% in pigs. Two metabolites were traced from the urine of calves but not from pigs.

Abadia *et al.* (1994) studied the pharmacokinetics of ciprofloxacin following i.v. administration in dogs using three doses (2.5, 5 and 10 $mg.kg^{-1}$). The elimination half-life was 129-180 min. The $AUC_{0-\infty}$ for the three doses were 138.33, 314.24

Table 2. Disposition kinetics of ciprofloxacin in domestic animals and poultry

Species	Dose ($mg.kg^{-1}$)	Route	Pharmacokinetic Parameters				Reference
			$t_{1/2\beta}$ (h)	AUC ($\mu g.h.ml^{-1}$)	Cl_B ($ml.h^{-1}.kg^{-1}$)	$V_{d\,area}$ ($L.kg^{-1}$)	
Calves	2.8	i.v.	2.44	3.93	726	2.5	Nouws *et al.* (1988)
Piglets	3.0	i.v.	2.57	2.88	1038	3.83	Nouws *et al.* (1988)
Ponies	5	i.v.	2.63	4.83	1087.2	3.45	Dowling *et al.* (1995)
Sheep	7.5	i.v.	1.205	7.02	1080	1.89	Munoz *et al.* (1996)
	7.5	i.m.	3.08	3.4	-	-	Munoz *et al.* (1996)
Cow calves	5	i.v.	3.239	5.872	857.4	4.05	Kumar *et al.* (1997)
Buffalo calves	4	i.v.	3.54	5.86	731	3.61	Raina *et al.* (2000)
Lactating cows	10	i.v.	2.15	3.37	906	2.838	Jayakumar *et al.* (2000)

and 775.65 mg.min^{-1}/L. Less than 37% of the drug was excreted unchanged in urine. The pharmacockinetics was linear at all the three doses studied.

Dowling *et al.* (1995) investigated pharmacokinetics of ciprofloxacin in ponies after i.v. and oral administration (5 mg.kg^{-1}). Plasma half-life was 157.89 min. The oral bioavailability was as poor as 6.8%. Based on the pharmacokinetic data and MICs of equine pathogen s, the appropriate dosage was determined to be 5.32 mg.kg^{-1} at 12 h intervals.

Munoz *et al.* (1996) studied the disposition kinetics of ciprofloxacin in sheep after i.v. or i.m. administration at a dose of 7.5 mg.kg^{-1}. The elimination half-lives were 72 and 184 min after i.v. and i.m. administration, respectively. After i.m. administration, the absorption was fast with a C_{max} value of 0.69 µg.ml^{-1} attained in 31.93 min (t_{max}) and the bioavailability was 49%.

Atta and Sharif (1997) determined the pharmacokinetic parameters of ciprofloxacin in broiler chicken after i.v. and oral administration at a dose of 5 mg.kg^{-1} body weight. The elimination half-life was 540.63 min. The $AUC_{0\text{-}24h}$ was 78.04 and 55.51 µg.h.ml^{-1} for i.v. and oral routes, respectively, with the oral bioavailability of 70%. The C_{max} value was 4.67 µg.ml^{-1} and the t_{max} was 42.5 min.

After i.v. administration of ciprofloxacin in buffalo calves, the elimination half-life was 1.1 4 h and the AUC was 5.71 mg.min.L^{-1}. Alterations in dosage regimens of ciprofloxacin in renal and hepatic dysfunctions in buffalo calves have been suggested (Saini, 1998). Kumar *et al.* (1997) investigated the pharmacokinetics of ciprofloxacin in cow calves after i.v. administration.(5 mg.kg ^{1}). The drug was detectable upto 12 h in the plasma. The important pharmacokinetic parameters were : $t_{1/2\beta}$, 194.35 min, AUC, 352.34 µg.ml.min^{-1}; MRT, 4.01 h, Cl_B, 14.29 ml.kg^{-1}. min^{-1}. and $V_{d\,area}$, 4.05 L. kg^{-1}. Based on the pharmacokinetic parameters, a 12 h dosage interval with a 6.8 mg.kg^{-1} loading dose were suggested. In buffalo calves,

ciprofloxacin kinetics was studied after i.v. administration at 4 mg. kg^{-1} (Raina *et al.*, 2000). The pharmacokinetic parameters were comparable to those obtained in cow calves. A dosage regimen with 4.80 mg. kg^{-1} i.v. loading dose, with 12-hourly dosage schedule was recommended.

In lactating cattle, after a 10 mg.kg^{-1} i.v. dose of ciprofloxacin, the pharmacokinetic variables were : $t_{1/2\beta}$ was 129.33 min; AUC 198.66 µg. min. ml^{-1}, Cl_B, 15.10 ml. min^{-1}. kg^{-1} and the $V_{d\,area}$, 2.84 L.kg^{-1} (Jayakumar *et al.*, 2000).

2.7 Effect of endotoxin-induced fever on the pharmacokinetics of antimicrobial agents

van Miert (1976) reported higher plasma sulphonamide levels in endotoxin-induced febrile goats, however, at shock producing doses of endotoxin, the drug concentrations were significantly lower. Halkin *et al.*, (1981) observed a two-fold increase in the V_d and a prolonged $t_{1/2\beta}$ of gentamicin administered i.v. in febrile rabbits. Wilson *et al.* (1983) observed significant increase in serum concentration data (A, B, C_p^0) and significant decrease in V_d and V_c of gentamicin in horses given small doses of *E. coli* endotoxin. Wilson *et al.* (1984) studied the effects of endotoxin-induced fever on gentamicin disposition in adult ewes and reported significant differences in zero time intercept (A), distribution rate constant, C_p^0, V_p and V_c.

Kume and Garg (1986) observed significant changes in the values of C_p^0, A, K_{el} and V_{dSS} of chloramphenicol between normal and febrile goats after its i.v. administration. No such change was evident after i.m. administration.

Mody (1989) studied the pharmacokinetics of sulphadimidine in buffalo calves before and after *E. coli* endotoxin administration. There was a significant increase in V_d and Cl_B but C_p^0 and AUC were significantly decreased in febrile animals.

Jha *et al.* (1996) evaluated the influence of endotoxin-induced fever on the biokinetics of norfloxacin in goats and reported significant decrease in Cl_B, K_{el} and K_{12}/K_{21} ratio. Zeng and Fung (1997) studied the effect of induced *E. coli* infection on enrofloxacin kinetics in pigs after i.v., i.m. and oral administration. They reported higher values of elimination half-life, t_{max} and lower values of V_d and clearance in infected pigs.

van Gogh and van Miert (1977) did not observe any significant effect of febrile condition either on the absorption of sulphonamides or on the rate of their metabolism in young and adult dwarf goats.

Ahmad *et al.* (1994) studied the pharmacokinetics of gentamicin following single dose i.v. administration in normal and febrile goats. Blood serum concentrations were similar between febrile and normal goats. Parameters like V_d, Cl_B and V_c were not affected by induction of febrile condition.

Urinary excretion of nalidixic acid was studied after i.v. injection at a dose of 10 $mg.kg^{-1}$ in healthy and febrile goats (Patel *et al.*, 1995). The cumulative amount of nalidixic acid excreted within 4 h was significantly higher in febrile goats (59.31±1.45 mg) than in afebrile goats (32.45+3.37 mg).

Ahmad and Sharma (1997) reported the disposition kinetics of gentamicin in febrile goats following i.m. administration at 5 $mg.kg^{-1}$.Less of the drug was available in febrile goats. An increase in elimination half-life was observed in febrile goats (104.8 min) as compared to normal goats (88 min). However, Cl_B and $V_{d\,area}$ were not affected.

The pharmacokinetics of cefazolin with and without probenecid was studied in febrile goats (Roy *et al.*, 1999). Concurrent administration of probenecid decreased the Cl_B, Cl_R and Cl_H of cefazolin in febrile goats. Probenecid significantly increased the $t_{1/2\beta}$ value of cefazolin more than two-fold in febrile goats.

Singh *et al.* (1998) studied the effect of induced fever on the pharmacokinetics of oxytetracycline in crossbred calves. The $t_{1/2\beta}$ and $V_{d\,area}$ were slightly increased in febrile calves as compared to healthy animals.

Rao (1999) reported slightly higher persistance of enrofloxacin in plasma of febrile goats as compared to normal goats after intramuscular administration. The metabolism of enrofloxacin to ciprofloxacin was also reduced in febrile goats.

In crossbred bovine calves, minimum therapeutic concentration of enrofloxacin was maintained upto 4 h. Approximately 10% of the total dose was excreted in urine in 24 h (Ahangar and Srivastava, 2000).

2.8 Effect of probenecid on the disposition of antimicrobial agents

Probenecid, (p-[Dipropylsulfamoyl] benzoic acid), is an organic anion transport inhibitor, and is used to prolong the biological half life of some penicillins and third generation cephalosporins.

Probenceid altered many pharmacokinetic parameters of benzylpenicillin, ampicillin and cloxacillin in lactating sheep, with a slowing down of renal clearance of these penicillins and a decrease in milk penicillin concentration (Ziv and Sulman, 1974). In young calves (1-3 week old), probenecid administration resulted in higher concentrations in the serum of three oral cephalosporins, cefalexin, cefradine or cefatrizine and the serum antibiotic concentration were maintained for a longer period (Ziv *et al.*, 1979). After parenteral injection of probenecid at 1 and 2 g/calf, serum ampicillin concentrations were double as compared to calves where equal doses of ampicillin was injected alone. Serum antibiotic concentration >5 $\mu g.ml^{-1}$ was maintained upto 5-6 h in probenecid co-treated calves, as compared to 2-3 h, when ampicillin/amoxycillin was injected alone.

Guerrini *et al* (1985) reported the effect of probenecid given by i.v., i.m., or s.c. injection on cefotaxime pharmacokinetics in ewes. After i.v. injection,

probenecid increased the plasma half life three-fold to 0.94 h, and the AUC by approximately two-fold to 41.1 $\mu g.h.ml^{-1}$ and decreased the plasma clearance by 45%. By i.m. or s.c. route, probenecid reduced the renal clearance and total body clearance of cefotaxime. The absorption of cefotaxime after s.c. administration was only 40%. The elimination half-life of ceftriaxone administered at dose of 10 $mg.kg^{-1}$ (i.m.), was 116.8 min which was increased to 141.3 on co-administration of probenecid, almost similar to 145.0 min achieved by doubling the i.m. dosage of ceftriaxone to 20 $mg.kg^{-1}$ (Soback and Ziv, 1988). However, the elimination half-life of cefuroxime was not affected by the administration of probenecid in unweaned calves (Soback *et al.*, 1989). Similarly, probenecid (40 or 80 mg. kg^{-1}) did not produce any alternation in the terminal half-life or MRT of ceftazidime (10 $mg.kg^{-1}$) in unweaned calves (Soback and Ziv, 1989a).

In another study, Soback and Ziv, (1989b) investigated the effect of probenecid on cefoperazone. After i.m. administration of cefoperazone, (20 $mg.kg^{-1}$), the terminal half life increased to 257.3 min due to coadministration of probenecid while it was only 136.9 min when cefoperazone was given alone. MRT was also significantly increased to 264.5 min. in probenecid-coadministered animals as compared to 140.3 min. in animals given cefoperazone alone. Probenecid caused significant changes in the pharmacokinetics of ticarcillin in sheep. After i.m. administration, the pharmacokinetic values were : $t_{1/2\ ab}$, 8.08 min, $t_{1/2\beta}$, 0.96 h; C_{max}, 31.11 $\mu g.ml^{-1}$ at 0.5 h (t_{max}) and F (bioavailability), 0.82 : After coadministration of probenecid, the following values were reported : $t_{1/2ab}$, 33.9 min; $t_{1/2\beta}$, 2.66 h; C_{max}, 44.87 at 1.33 h and F, 1.25.

The pharmacokinetics of sparfloxacin, a newly developed fluoroquinolone, was not affected by probenecid (Shimada *et al.*, 1993). However, on the kinetics of T-3761, another novel fluoroquinolone, probenecid induced increases in the elimination half-life (2.1 times), AUC (3.1 times) and decreases in ß (0.44 times) and total body clearance (0.35 times) in a study conducted in rabbits.

The effect of probenecid was determined on the pharmacokinetics of ciprofloxacin in humans (Jaehde *et al.*, 1995). Following a single dose of ciprofloxacin (200 mg. i.v.), with and without multiple oral administration of probenecid in two different groups of human subjects, it was found that the plasma AUC, $t_{1/2\beta}$ of ciprofloxacin and its metabolite MI were increased significantly, while urinary recovery, renal clearance and total body clearance were decreased. Saliva, sweat and tear exposures were elevated ($P<0.05$). The metabolite ratio of MI was increased significantly. MRT of ciprofloxacin was also significantly enhanced.

2.9 Effect of piperine on the bioavailability of co-administered drugs

In Ayurveda, ginger (*Zingiber officianalis* Rose), black pepper (*Piper nigrum* Linn.) and long pepper (*Piper longum* Linn.) together constitute the trikatu, a word meaning three acids. Trikatu is an essential ingredient of numerous ayurvedic prescriptions and formulations, used for a wide range of diseases (Atal *et al.*, 1985). Of these three agents, atleast the two peppers are found to contain an active alkaloidal principle piperine (1-piperoyl piperidine). The crude extract from these plants or the pure principle piperine have been shown to stimulate respiration and induce convulsions in mice (Piyachaturawat *et al.*, 1983). It was also reported to inhibit implantations, cause abortion and delay labor in mice. However, the most important activity of piperine that has aroused significant interest has been its property of enhancing the efficacy of other drugs. The first mention of such an activity was given by Bose (1928), who indicated that addition of long pepper to vasaka leaves (Adhatoda vasica) increases the efficacy of anti-asthmatic property of the latter.

Atal *et al.* (1981) confirmed the observation and indicated that the enhancement of efficacy is due to the enhancement of bioavailability of vasicine, an alkaloid from vasaka leaves. Other studies have also reported enhancement of blood levels of drugs like rifampicin when coadministered with piperine or trikatu (Zutshi *et al.*, 1985). Bano *et al.* (1987) observed significant increases in the C_{max}

and AUC of phenytoin in human volunteers when coadministered with prior multiple doses of piperine (20 mg OD x 7 days p.o.).Piperine has also been reported to enhance the AUC, C_{max}, t_{max} and $t_{1/2\,\beta}$ of oxyphenbutazone besides significantly enhancing its antiinflammatory activity in rats. Piperine was shown to enhance the absorption and slow down the degradation of oxyphenbutazone, thus accounting for the enhanced bioavailability of oxyphenbutazone (Majumdar *et al.*, 1999).

On pentobarbitone induced hypnosis, which is an indirect measure of hepatic microsomal mediated in activation, piperine potentiated the pentobarbital sleeping time which also correlated with increased levels of pentobarbitone in blood and brain of rats pretreated with piperine at an oral dose of 10 $mg.kg^{-1}$ (Majumdar *et al.*, 1990).

While trying to explain the bioavailability enhancing effects of piperine, Atal *et al.* (1981) opined that this effect may be due to i) promoting absorption from the gastrointestinal tract; ii) protecting the drug from being metabolised/ oxidised; or iii) a combination of these two mechanisms. Atal *et al.* (1985) provided conclusive evidence that piperine is a potent non-specific inhibitor of drug metabolism. When added *in vitro* to rat preparation, piperine was shown to be a non-specific inhibitor of drug metabolising enzyme, by inhibiting both 3-methylcholanthrene-induced and phenobarbital-induced microsomal enzymes.

In vivo, piperine at a dose of 125 mg/kg p.o. caused a maximal inhibitory effect on hepatic metabolism. Piperine also enhanced hexobarbital-induced sleeping time in rats after both oral and intraperitoneal administration. Reen and Singh (1991) further demonstrated that piperine *in vitro* strongly inhibits both constitutive and inducible monooxygenases in the pulmonary tissue of rats, which however was much lower in guinea pig pulmonary monooxygenases. Khajuria *et al.* (1998), on investigating the effect of piperine on absorption using everted intestinal sacs, reported that piperine diffused passively constituting a nonsaturable absorption

kinetics free of any rate limiting factor. They also suggested that it may act as an apolar molecule due to its lipophilic nature. Due to its easy partitioning and interaction with the membrane, it may modulate the membrane dynamics which can induce increase in absorptive area leading to efficient permeation of coadministered drugs through membranes. Thus, apart from decreased metabolism, enhanced intestinal absorption of orally administered drugs could be a contributing factor for increased bioavailability of drugs co-administered piperine.

2.10 Correlation of pharmacokinetic and pharmacodynamic variables for successful antimicrobial therapy

The optimal dosage regimen of an antimicrobial agent is arrived at keeping in mind the necessity to achieve successful clinical outcome with no deleterious side effects and avoidance of emergence of bacterial resistance. In the last decade, considerable attention has been directed on the elucidation of a suitable correlation between the pharmacokinetic parameters and the pharmacodynamic variables in antimicrobial therapy.

The pharmacokinetic parameters quoted frequently to be important for a good correlation are the C_{max} (peak serum concentration), AUC (Area under the plasma concentration-time curve) and T > MIC (the time that biological fluid drug concentration exeeds the MIC values against the organism in question).

The pharmacodynamic parameters used to measure or predict the antimicrobial action are :

1. *In vitro* susceptibility tests : based on dilution or diffusion methods such as

   a) agar disc diffusion tests which provide qualitative data.

   b) Minimum inhibitory concentration (MIC) determined by doubling dilution technique and is defined as the lowest concentration of drug that prevents visible bacterial growth.

c) Minimum bactericidal concentration (MBC) measures the killing action on bacteria and is defined as the lowest concentration of drug which kills at least 99.9% of organism in the original inoculum over a specific time period.

2. Serum bactericidal titre concentration is the highest dilution of a serum sample containing antimicrobial drug which kills at least 99.9% of bacteria in the original inoculum.

3. Kinetics of bacterial killing : measures the extent and rate of bacterial killing which may be concentration dependent or concentration independent (time dependent killing).

4. Post-antibiotic effect (PAE) : It is the period of persistent suppression of bacterial growth following complete removal of the antibacterial drug. Bactericidal agents have been known to exert postantibiotic effect. For example, penicillins have been known to exert PAE for upto 2.5 h while those agents which could inhibit protein or nucleic acid synthesis induce a longer suppression (Vogelman and Craig, 1986). Fluoroquinolones are able to induce a PAE of about 2 h in streptococci and pneumococci and of 0.9 to 2.4 h in some Gram-negative bacteria (Odenholt-Tornqvist *et al.*, 1992; Odenholt- Tornqvist and Bengtsson, 1994). The PAE has been suggested as one of the explanations for successful therapy even after intermittent dosages of antibiotics.

5. Post antibiotic sub-MIC effect (PASME) : It is the further period of inhibition of bacterial growth when the bacteria are reexposed to sub-MICs of the same antibacterial agent during the post-antibiotic phase. The sub-MIC phase, which follows the supra-inhibitory concentration of a drug, may alter bacterial cell wall and growth and enhance the susceptibility of the bacteria to phagocytosis (Lorian and Gemmel, 1991; McDonald *et al.* 1981; Rapon *et*

al. 1990 and van der Auwera, 1991). Beta-lactams, vancomycin and roxithromycin exert a very strong PASME on gram-positive cocci (Odenhold-Tornqvist, *et al.*, 1989, 1991, 1992), but a very short or negligible PASME has been noted for drugs that exert no PAE e.g., beta-lactams on gram-negative bacilli (Odenholt-Tornqvist *et al.*, 1991). Sparfloxacin, a fluoroquinolone exhibits a PASME of approximately 6 h against streptococci and pneumococci. For benzylpenicillin, the PASME against ß-hemolytic streptococci was 2-3 h longer than PAE (Lowdin *et al.*, 1993).

Other pharmacodynamic variables used for prediction of antibacterial activity of drugs include post-antibiotic leucocyte enhancement (PALE), *ex vivo* bactericidal activity and *in vivo* antibacterial activity.

In the various attempts to integrate and correlate pharmacodynamic and pharmacokinetic variables of antibacterials, towards achieving successful clinical outcome, the following approaches have attracted considerable interest.

1. Peak serum/plasma concentration-to-MIC ratio (C_{max}/MIC) :

A C_{max}/MIC ratio of 8 was found to be necessary for prevention of bacterial regrowth within 24 h whereas a ratio of 10 was associated with optimum bactericidal activity of aminoglycosides. A C_{max}/MIC ratio of 20 was found to result in a greater successful therapy following once daily administration of lomefloxacin than divided doses yielding the same AUC, in a neutropenic rat model of *P. aeruginosa* (Drusano *et al.*, 1993).

2. Area under the inhibitory serum concentration curve (AUIC) :

This is another parameter which has been highly correlated with successful therapy for antibiotics which produce concentration-dependent killing. AUIC can be determined as the ratio of AUC-to-MIC. Clinical studies with ciprofloxacin have established AUIC values of> 125, 125-250 and >250 to result in an eradication

time of >32, <7 and <2 days, respectively. Dosage regimens yielding AUIC of at least 100 may reduce the development of bacterial resistance (Thomas *et al.*, 1998). Nightingale *et al.* (2000), suggested that for effective eradication and good clinical outcome with fluoroquinolones would require AUIC of >100 and >30 for gram-negative and gram-positive organisms, respectively.

3. Time the serum or biological fluid concentration exceeds MIC (T > MIC) :

For antibiotics that produce time dependent killing, such as beta-lactams and erythromycin, clinical outcomes have been correlated with the amount of time their concentration exceeded MIC values (Vogelman *et al.*, 1988).

Fluoroquinolones are bactericidal agents, which like aminoglycosides, produce concentration-dependent killing. This necessitates the use of dosage regimens that would yield high peak concentrations intermittently (Dudley, 1991; Nightingale *et al.*, 2000). Considering the PAE and the PASME, it is not necessary to maintain the plasma concentration of fluoroquinolones above MIC throughout the dosing interval. The integrated parameters C_{max}/MIC and AUIC have been shown to be valid for these agents. For enrofloxacin, the optimal dosage regimen may be designed to maximise serum drug concentration and not the time the drug concentrations remain above MIC (Meinen *et al.*, 1995).

MATERIALS AND METHODS

3.1. Experimental animals

The study was conducted in non descript female goats procured from the Livestock Production Research Unit, IVRI, Izatnagar. Before the start of the experiment, they were examined clinically to rule out the possibility of any disease. They were housed in animal shed with concrete floor and were maintained on concentrate, green fodder and dry grass. Water was provided *ad libitum*. A minimum washout period of 15 days was maintained between each trial.

3.2 Drugs and chemicals

Injectable formulation of enrofloxacin (Enrocin [10%]) supplied by M/s Ranbaxy Laboratories (P) Ltd., New Delhi, was used in the study. Pure ciprofloxacin HCl gifted by Cipla Pharmaceuticals, Mumbai, was used to prepare a 3% solution of ciprofloxacin in sterilized distilled water. For external standards, pure technical

grade enrofloxacin and ciprofloxacin generously gifted by M/s Intas Pharmaceuticals, Ahmedabad, and M/s Cipla Ltd., Mumbai, were used.

Probenecid and piperine were obtained from Sigma, St. Louis, U.S.A. and *E. coli* endotoxin (Lipopolysaccharide) was obtained from Difco Laboratories, USA. Heparin was purchased from SISCO Research Laboratories, Mumbai.

For HPLC analysis, HPLC grade acetonitrile and methanol were procured from Qualigens Fine Chemicals Ltd., Mumbai. Water for HPLC was obtained by Millipore water purification system and was filtered using 0.2 μm filter prior to use. All other chemicals used in this study were of analytical grade.

3.3 Plan of Work

The work was undertaken in eight phases. The plan of work is summarised in Table 3.

Table 3 : Plan of work

Phase I	Study	Animal Number
I.	Plasma levels and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin in goats after single intravenous administration of enrofloxacin (5 $mg.kg^{-1}$)	1,2,3,4,5
II.	Plasma levels and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin in goats after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$)	1,2,3,4,5
III.	Plasma levels and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin	1,2,3,4,5

	in febrile goats after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$)	
IV.	Plasma levels and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin in goats after coadministration of enrofloxacin (5 $mg.kg^{-1}$, s.c.) and piperine (2 $mg.kg^{-1}$, s.c.)	1,2,3,4,5
V.	Plasma levels and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin in goats after coadministration of enrofloxacin (5 $mg.kg^{-1}$, s.c.) and probenecid (40 $mg.kg^{-1}$, s.c.)	1,2,3,4,5
VI.	Plasma levels and pharmacokinetics of ciprofloxacin in goats after single intravenous administration of ciprofloxacin (7.5 $mg.kg^{-1}$)	1,2,3,4,6
VII.	Plasma levels and pharmacokinetics of ciprofloxacin in goats after single subcutaneous administration of ciprofloxacin (7.5 $mg.kg^{-1}$)	1,2,3,4,6
VIII.	Plasma levels and pharmacokinetics of ciprofloxacin in goats after coadministration of probenecid (40 $mg.kg^{-1}$ s.c.) and ciprofloxacin (7.5 $mg.kg^{-1}$ s.c.)	1,2,3,4,6

3.4 Induction of febrile state

Febrile condition was induced in goats by administration of *Escherichia coli* endotoxin (lipopolysaccharide, LPS). Endotoxin solution in pyrogen-free normal saline (10 $\mu g.ml^{-1}$) was injected intravenously in goats at a dose of 0.2 $\mu g.kg^{-1}$ body weight. Febrile response was monitored by recording of rectal temperature. A rise of 1-1.5°F in rectal temperature was considered as indication of febrile condition. Once the febrile state set in, enrofloxacin was administered.

To maintain the febrile condition for 12 h, a second dose of endotoxin at half the initial dose (0.1 $\mu g.kg^{-1}$) was given at 5 h.

3.5. Preparation of probenecid solution

2 g of probenecid was dissolved in 8 ml of 1N sodium hydroxide under constant stirring. The pH was then adjusted to 7.5-8.0 with 1N HCl. The final volume was made up with pyrogen-free distilled water to yield a 12.5% solution. This solution was injected subcutaneously at a dose of 40 $mg.kg^{-1}$ in goats immediately prior to subcutaneous administration of enrofloxacin (phase V) or ciprofloxacin (phase VIII).

3.6. Preparation of Piperine solution

A 2.5% solution of piperine was prepared in dimethylsulfoxide and this was injected subcutaneously at a dose of 2 $mg.kg^{-1}$, immediately prior to administration of enrofloxacin (phase IV).

3.7. Administration of drugs and collection of blood samples

In phase I and phase VI of the study, enrofloxacin and ciprofloxacin, respectively, were injected into the jugular vein after shaving and cleaning the site.

In other phases of the study involving subcutaneous administration of drugs, enrofloxacin/ciprofloxacin were injected subcutaneously in the right fore flank region. When probenecid/piperine was coadministered, they were injected subcutaneously in the opposite anatomical region immmediately prior to administration of enrofloxacin/ciprofloxacin.

Blood samples (2-3 ml) were collected by jugular venepuncture into heparinised tubes immediately before and at 0.033, 0.083, 0.167, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 10.0, 12.0, 24.0 and 48.0 h after enrofloxacin

or ciprofloxacin administration. In intravenous study, care was taken to collect blood from the contralateral jugular vein. Blood was centrifuged at 950 x g for 20 minutes to separate the plasma. The plasma samples were immediately stored at –20°C until assayed.

3.8. Analytical Procedure

Assay of enrofloxacin and/or ciprofloxacin in plasma.

3.8.1 Sample Extraction

Sample extraction was performed according to the method of Nielsen and Hansen (1997) as described below.

To 0.5 ml of plasma, 0.75 ml of acetonitrile was added in the ratio of 1:1.5 in a test tube. After vortex-mixing at high speed for 15 sec, the tube was subjected to centrifugation for 10 min at 950 x g. The clear supernatant thus obtained, was transferred to a tube and twice the volume of HPLC grade water was added. The aliquot was then filtered through a 0.22 µm cellulose acetate membrane filter and a 20 µl of filtrate was injected into the HPLC system.

3.8.2 High Performance Liquid Chromatography

The method developed by Kung *et al.* (1993) was used with some modification. The HPLC system (Shimadzu Corporation, Kyoto, Japan) comprised of LC-10AT double plunger pump, Rheodyne manual loop injector with a 20 µl loop, column oven CTO-10 AS vp, SPD-10A UV-vis detector/RF-10 AXL flourescent detector and a software chromatopak for data analysis. Separation of enrofloxacin and its metabolite, ciprofloxacin, was achieved using a reverse phase column [particle size 5 µm; 4.6 x 250 mm, Shimadzu Corporation, Japan] as stationary phase and a mixture of acetonitrile-methanol-water (17:3:80, v/v/v), containing 0.4% phosphoric acid (85%, v/v) and triethylamine as mobile phase (pH 3). The

flow rate of the mobile phase was adjusted to 0.8 ml. min^{-1}. Chromatography was performed at 40°C with detection at 278 nm. For flourescent detection, the excitation wavelength was set at 278 nm and the emission wavelegnth at 440 nm. There were no interfering substances in the plasma at the retention times of enrofloxacin and ciprofloxacin. The data collected were analysed with a chromatopak software, taking into account the peak areas/peak heights of the drug.

3.8.3 Analytical Recovery and Precision

The assay was performed by external standard method. A stock solution of 1 $mg.ml^{-1}$ enrofloxacin was prepared in 0.1 N NaOH, whereas to prepare a stock solution of ciprofloxacin base (1 $mg.ml^{-1}$), 1.12 mg of ciprofloxacin hydrochloride was dissolved in 1.0 ml of HPLC grade water. From these stock solutions, working standards were prepared daily.

Analytical recovery was determined by adding enrofloxacin and ciprofloxacin to fresh pooled plasma obtained from drug untreated goats or to a solution of mobile phase to yield concentrations of 0.1 and 1 $\mu g.ml^{-1}$ and then analysed. Both plasma/ mobile phase standards were treated as described above. Recovery was calculated by dividing the peak areas obtained for plasma based standards by those obtained from standards in mobile phase. Four determinants were made for each concentration. Per cent recovery was calculated according to the formula :

$$\% \text{ recovery} = \frac{N\sum_{xy} - (\sum x)(\sum y)}{N\sum X^2 - (\sum x)^2}$$

where,

x = known amount of drug added (external standard)

y = amount of drug found by the assay method

N = number of observations

The recovery data of enrofloxacin and its metabolite, ciprofloxacin, are summarized in Table 4.

Table 4 : Recovery of enrofloxacin and ciprofloxacin in goat plasma

	Plasma			
	Enrofloxacin		Ciprofloxacin	
Concentration spiked ($\mu g.ml^{-1}$)	1.0	0.1	1.0	0.1
Concentration found (Range, $\mu g.ml^{-1}$)	0.837-1.085	0.068-0.095	0.882-1.072	0.0705-0.091
Mean±SE ($\mu g.ml^{-1}$)	1.006±0.117	0.08±0.012	0.972±0.097	0.083±0.0083
Coefficient of Variation %	8.4	13.8	10.1	10.0
Recovery %	**101**		**98.7**	

Intra-day variation was determined by assaying two standard plasma samples (0.1, 1.0 $\mu g.ml^{-1}$) four times each. Inter-day variation was also determined by assaying two standard plasma samples (0.1, 1.0 $\mu g.ml^{-1}$) on four occasions at least 24 h apart. The summary of results is presented in Table 5.

Table 5 : Intra- and inter-day assay precision of the HPLC assay for enrofloxacin and ciprofloxacin

Sample	Concentration ($\mu g.ml^{-1}$)	Intra- day assay CV (%)		Inter- day assay CV (%)	
		ENR	CIP	ENR	CIP
Plasma	1.0	4.2	9.1	4.4	7.6
	0.1	13.4	4.8	12.8	9.0

3.8.4 Preparation of Standard Curves

Separate plasma standards of enrofloxacin (0.01 to 8.0 $\mu g.ml^{-1}$) and ciprofloxacin (0.01 to 8.0 $\mu g.ml^{-1}$) were used. Working plasma standards were prepared from stock solutions of enrofloxacin and ciprofloxacin after diluting with pooled goat plasma. These standards of known concentrations were analysed as described above. Peak areas/peak heights obtained were plotted against concentrations of standards to obtain standard curves for enrofloxacin and ciprofloxacin separately. The standard curves of enrofloxacin and ciprofloxacin were linear in the range of 0.01 to 8.0 $\mu g.ml^{-1}$,. The lowest concentration of standard routinely used was 0.01 $\mu g.ml^{-1}$. The minimal limit of detection for both the drugs was 0.01 $\mu g.ml^{-1}$.

3.8.5 Quantification

The concentrations of enrofloxacin and ciprofloxacin in the plasma samples were determined by substituting the respective peak areas/peak heights in the linear regression formula after calibration of standard curves.

$Y = a + bx$

Conc. $(Y) = RF_2 + RF_1$ x peak area/peak height

RF_1 = Response factor 1

RF_2 = Response factor 2

3.9 Pharmacokinetic Analysis

3.9.1 Compartmental analysis

Plasma concentration versus time data of enrofloxacin and ciprofloxacin obtained during phases I to V of the study were utilized for calculating various pharmacokinetic parameters in female goats with an iterative least-squares nonlinear

Shimadzu CLASS-VP V5.03 *Area % Report* **Page 1 of 1**

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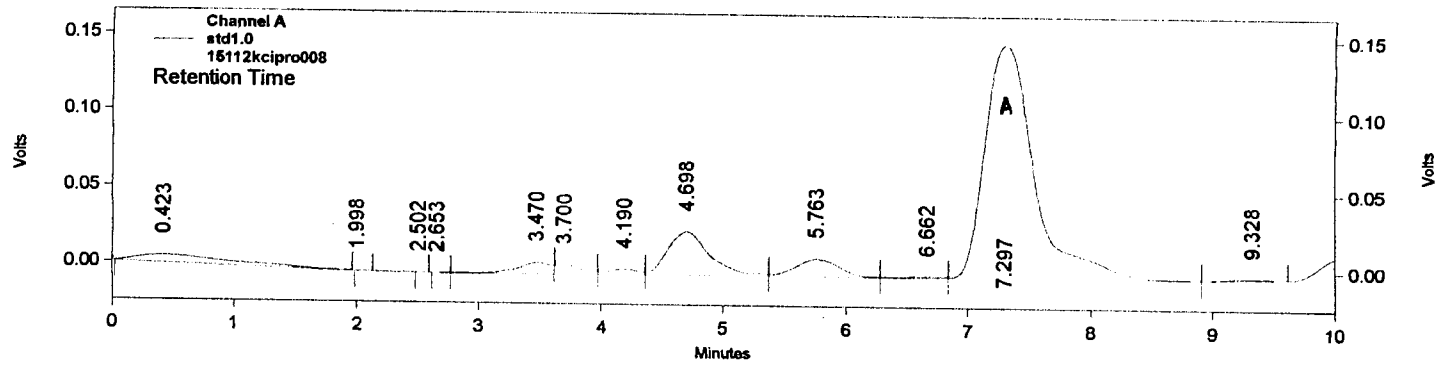

Shimadzu CLASS-VP V5.03 *Area % Report* **Page 1 of 1**

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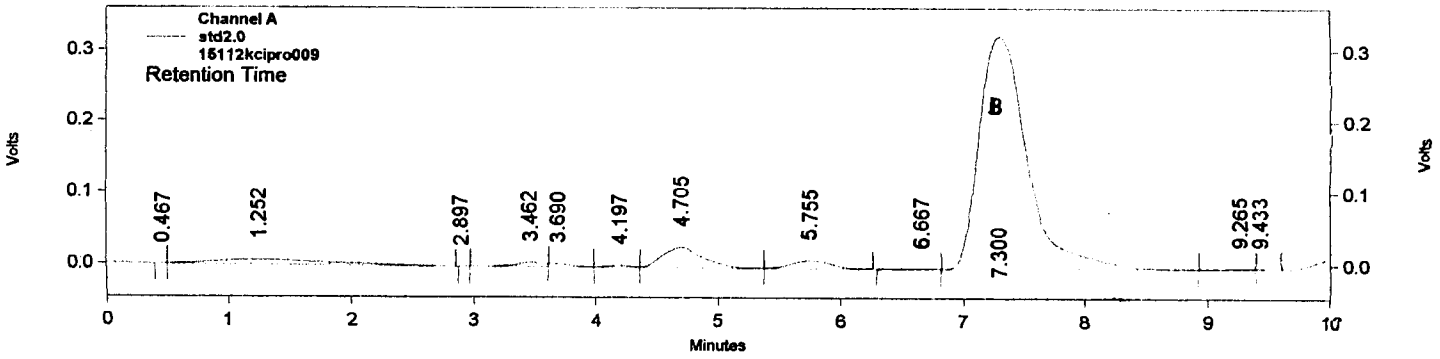

HPLC Chromatogram of Ciprofloxacin

A. Ciprofloxacin 1.0 µg/ml.
- Peak area - 4298779
- Peak height -151774

B. Ciprofloxacin 2.0 µg/ml.
- Peak area - 8887318
- Peak height -324782

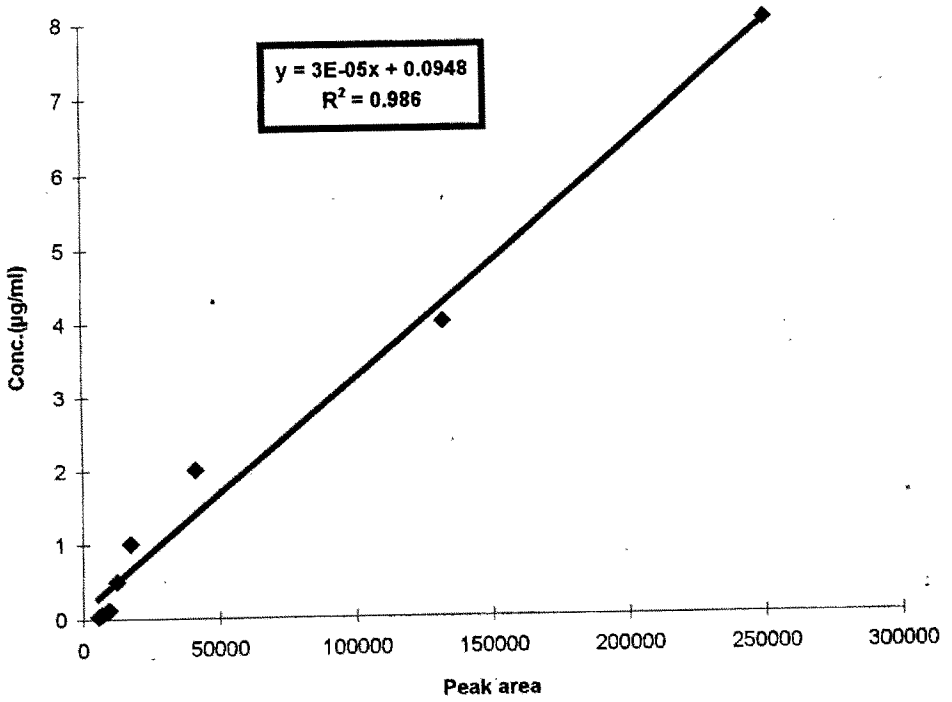

Fig. 1 Standard curve of Enrofloxacin - UV detector

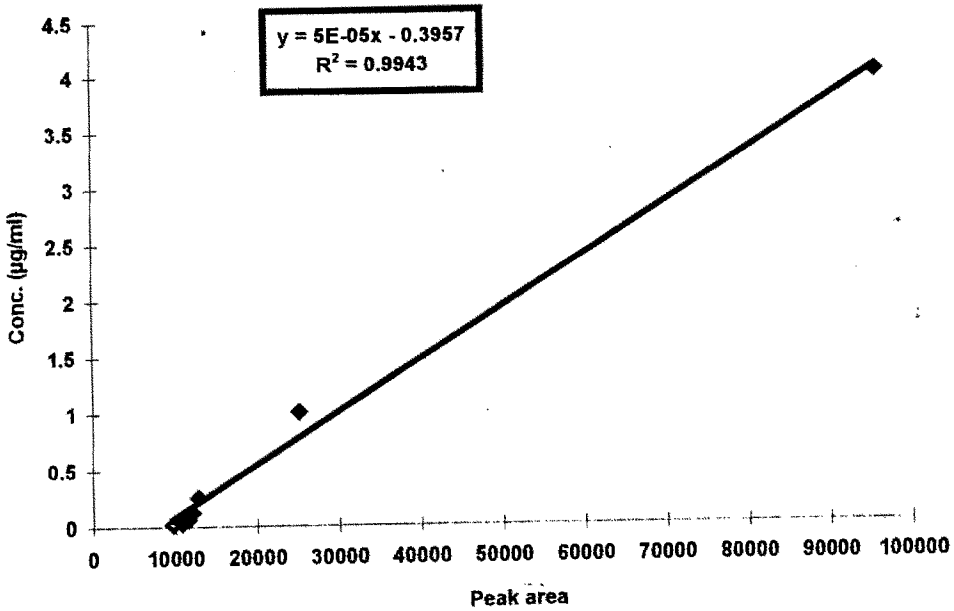

Fig. 2 Standard curve of ciprofloxacin - UV detector

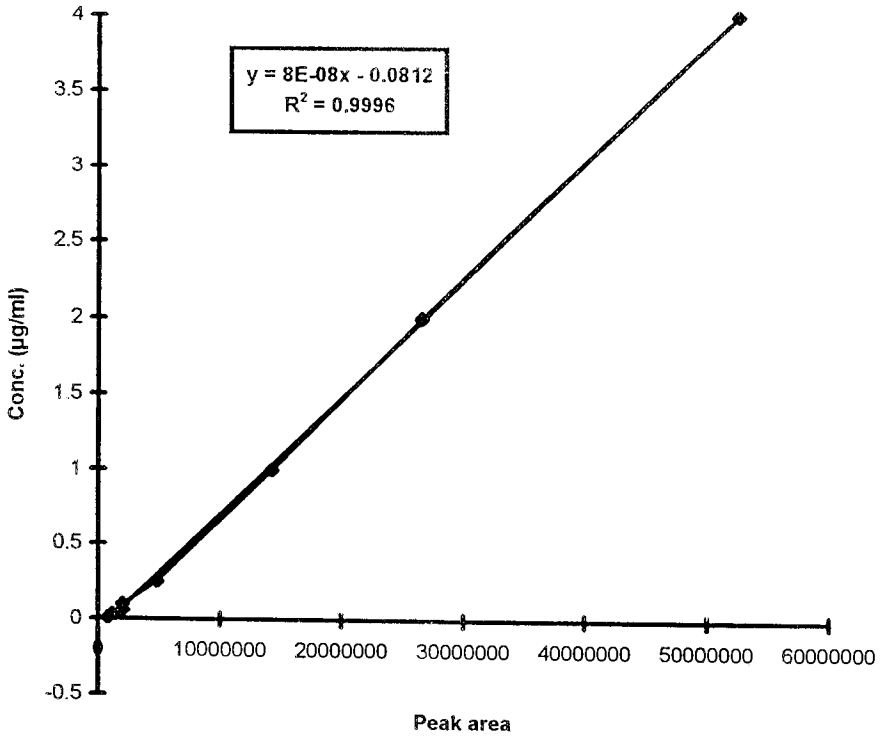

Fig. 3 Standard curve of Enrofloxacin - flourescent detector

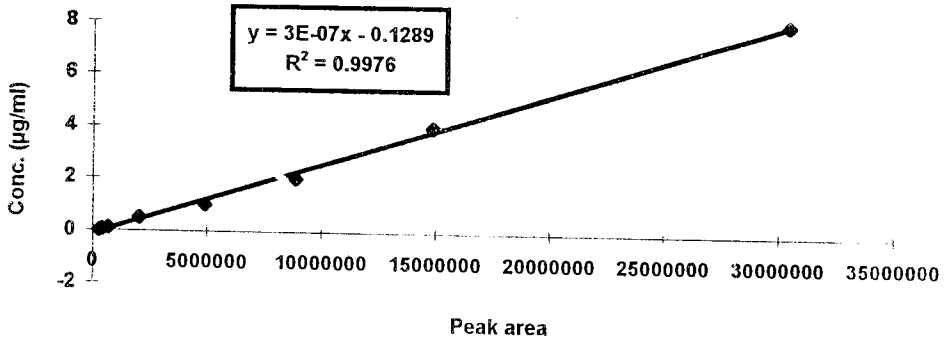

Fig. 4a Standard curve of Ciprofloxacin - flourescent detector

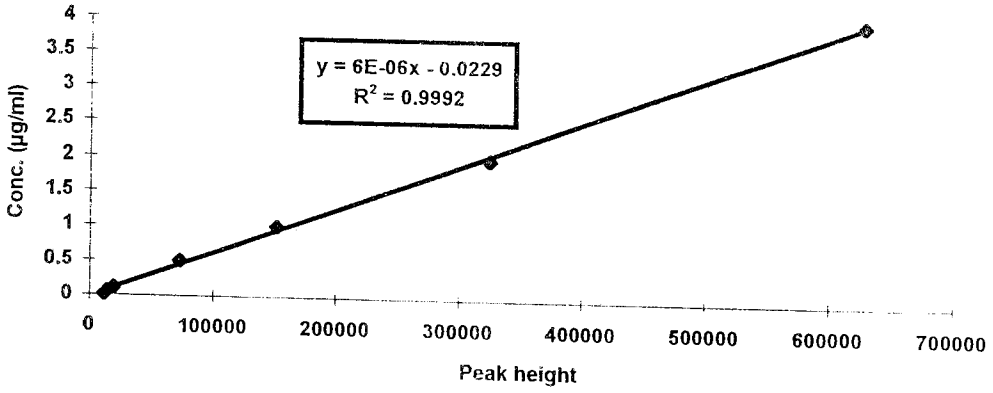

Fig. 4b Standard curve of ciprofloxacin - flourescent detector

regression programme for personal computer software, 'PHARMKIT' and according to the methods described by Baggot (1977) and Gibaldi and Perrier (1982).

a) B and ß, the regression coefficients for elimination phase of the plasma concentration versus time curve were calculated by the method of least squares.

b) A and α, the regression coefficients for distribution phase of the plasma concentration versus time curve were calculated by the method of residual yields (Two-compartment open model).

c) A' and ka, the regression coefficients for absorption phase of the plasma concentration versus time curve were calculated by the method of residuals yields (One-compartment open model).

d) k_f, the regression coefficient for metabolite formation phase of the plasma concentration versus time curve was calculated by the method of residual yields.

e) $t_{1/2\ ka}$, absorption half-life; $t_{1/2\ kf}$, metabolite formation half-life; $t_{1/2\alpha}$, distribution half-life and $t_{1/2ß}$, elimination half-life of the drug and/or the metabolite were also determined.

(i) $t_{1/2\,Ka} = \frac{0.693}{Ka}$ (ii) $t_{1/2\,Kf} = \frac{0.693}{K_f}$

(iii) $t_{1/2\alpha} = \frac{0.693}{\alpha}$ (iv) $t_{1/2ß} = \frac{0.693}{\beta}$

(f) Cp^o, the theoretical concentration of the drug in plasma at zero time, $Cp^o = A+B$

(g) $AUC_{(0-\infty)}$, the total area under the plasma drug/metabolite concentration-time curve was calculated by trapezoidal method.

(h) $AUMC_{(0-\infty)}$, the area under the first moment of the plasma drug/ metabolite concentration-time curve was calculated by trapezoidal method.

(i) K_{el}, the elimination rate constant of the drug from central compartment.

$$K_{el} = \frac{Cp^{o}}{Area}$$

(j) K_{21}, the rate constant of transfer of drug from tissue to the central compartment,

$$K_{21} = \frac{A\beta + B\alpha}{Cp^{o}}$$

(k) K_{12}, the rate constant of transfer of drug from central to tissue compartment,

$$K_{12} = \alpha + \beta - K_{el} - K_{21}$$

(l) F_c, the fraction of the drug in the central compartment,

$$Fc = \frac{\beta}{K_{el}}$$

(m) $V_{d\,area}$, the volume of distribution of drug based on area,

$$V_{d\,area} = \frac{dose}{\beta x\ area}\ (i.v.\ study)$$

$$V_{d\,area} = \frac{Dose \times F}{\beta \times area}\ (s.c.\ study)$$

(n) V_c, the volume of distribution of drug in the central compartment

$$V_c = \frac{Dose}{A + B}$$

(o) V_p, the volume of distribution of drug in the peripheral compartment,

$$V_p = \frac{V_c . K_{12}}{K_{21}}$$

(p) V_{dss}, the volume of distribution of drug at steady-state,

$$V_{dss} = \text{Dose} \times \frac{AUMC}{(AUC)^2}$$

(q) $Cl_B = \frac{\text{Dose}}{AUC}$

(r) T/P ratio, the tissue to plasma ratio of the drug,

$$\text{T/P ratio} = \frac{1}{F_c} - 1$$

(s) MRT, the mean residence time,

$$MRT = \frac{AUMC}{AUC}$$

(t) MAT, the mean absorption time,

$$MAT = MRT_{n.v.} - MRT_{i.v.}$$

(u) F, the fraction of drug absorbed after extravascular administration (bioavailability)

$$F = \frac{AUCextravascular}{AUCintravenous} x100$$

(v) MR, the metabolite ratio,

$$MR = \frac{AUC\ metabolite}{AUC\ parent\ drug}$$

3.9.2 Non compartmental analysis

The plasma drug concentration-time data of ciprofloxacin of in each animal of phases VI, VII and VIII were analysed by non-compartmental techniques based on statistical moments theory (Yamaoka *et al.*, 1978)

Using the trapezoidal rule, AUC_{0-t} and $AUMC_{0-t}$, where 't' is the time at which the last drug concentration was measured, were obtained. The terminal rate constant (β) was obtained using the linear regression analysis of the last 8-12 concentrations in a semilogarithmic plot of the concentration-time curve. The AUC and AUMC were extrapolated to infinity using the β and the last measured concentration.

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C^{last}}{\beta}$$

$$AUMC_{0-\infty} = AUMC_{0-t} + \frac{C^{last} \times t^{last}}{\beta} + \frac{C^{last}}{\beta^2}$$

b) The mean residence time (MRT) was calculated according to the equation

$$MRT = \frac{AUMC}{AUC}$$

c) The volume of distribution, $V_{d\ area}$ was calculated according to the equation.

$$V_{d\ area} = \frac{Dose \times F}{\beta \times AUC_{0-\infty}}$$

where 'F' is the fraction of drug absorbed.

d) The total body clearance was calculated as

Cl_B = Dose/AUC

e) The elimination half life ($t_{1/2\beta}$) resulted from :

$$t_{1/2\beta} = \frac{\ln 2}{\beta}$$

The C_{max} (maximum plasma drug concentration) and t_{max} (time to reach maximum plasma drug concentration) were obtained from the observed data.

3.10. Statistical Analysis

Statistical analysis of the data was performed by using computer software Microsoft Excel. The plasma concentrations were expressed as mean ± SE. The pharmacokinetic parameters of enrofloxacin/ciprofloxacin were expressed as median (range). Statistical differences between normal and febrile; normal and probenecid-treated and normal and piperine-treated goats were tested by Student's 't' test for plasma concentrations (Snedecor and Cochran, 1980) and by Wilcoxon's signed ranks testfor the pharmacokinetic parameters (Conover, 1980). A value of $P<0.05$ was considered as significant.

RESULTS

4.1 Plasma levels and pharmacokinetics of Enrofloxacin (ENR) and ciprofloxacin (CIP) after single intravenous administration of ENR (5 mg.kg^{-1})

The plasma levels of ENR and CIP at various time intervals in the five goats are presented in Tables 6 and 7, respectively. Mean plasma values of both ENR and CIP are represented graphically in Fig. 5. The mean peak plasma level of ENR was (11.66 ± 1.48 μg.ml^{-1}) at 0.033 h which declined rapidly to 2.315 ± 0.314 μg.ml^{-1} at 1 h. ENR could be detected in the plasma upto 8 h.

Ciprofloxacin appeared in plasma (0.199 ± 0.059 μg.ml^{-1}) of all the animals within 0.083 h after intravenous administration of enrofloxacin (5 mg.kg^{-1}). Ciprofloxacin attained a peak plasma concentration (C_{max}) of 1.27 (0.818-2.13) μg.ml^{-1} at 1 h (t_{max}) and its levels were detected in the plasma up to 5-6 h.

Table 6. Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin after single intravenous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	8.954	7.673	13.175	13.795	15.059	11.66 ± 1.48
0.083	7.599	6.304	9.070	7.601	8.413	7.793 ± 0.464
0.167	6.004	5.336	7.847	5.754	8.345	6.657 ± 0.602
0.25	5.393	4.837	6.570	4.616	7.169	5.717 ± 0.496
0.5	3.531	3.453	3.722	4.177	5.676	4.112 ± 0.411
0.75	2.831	2.159	3.137	4.064	3.993	3.236 ± 0.360
1.0	1.770	1.518	2.219	3.022	3.045	2.315 ± 0.314
1.5	1.307	0.829	2.080	1.340	2.180	1.547 ± 0.550
2	0.979	0.683	1.659	1.230	1.473	1.205 ± 0.173
3	0.498	0.224	1.452	0.920	0.562	0.731 ± 0.211
4	0.276	0.143	0.243	0.639	0.380	0.336 ± 0.085
5	0.209	0.042	–	0.628	0.256	0.227 ± 0.111
6	0.182	–	–	0.480	0.123	0.157 ± 0.088
7	0.138	–	–	0.240	0.108	0.097 ± 0.045
8	–	–	–	0.146	–	0.029 ± 0.029

Table 7. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after single intravenous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	–	–	0.070	–	–	0.014 ± 0.014
0.083	0.381	0.291	0.152	0.082	0.089	0.199 ± 0.059
0.167	0.531	0.802	0.257	0.257	0.198	0.409 ± 0.114
0.25	0.796	0.850	0.320	0.312	0.364	0.528 ± 0.121
0.5	0.808	0.936	0.349	0.438	0.520	0.610 ± 0.112
0.75	1.435	1.270	0.638	0.522	0.801	0.933 ± 0.179
1.0	1.576	1.014	0.961	0.762	1.201	1.103 ± 0.137
1.5	1.476	0.830	1.778	0.818	0.875	1.159 ± 0.202
2	1.012	0.127	2.133	0.627	0.612	0.902 ± 0.338
3	0.809	0.084	1.125	0.373	0.377	0.554 ± 0.184
4	0.647	0.020	0.640	0.134	0.190	0.326 ± 0.132
5	0.208	–	0.214	0.057	0.035	0.103 ± 0.045
6	–	–	0.020	–	–	0.004 ± 0.004

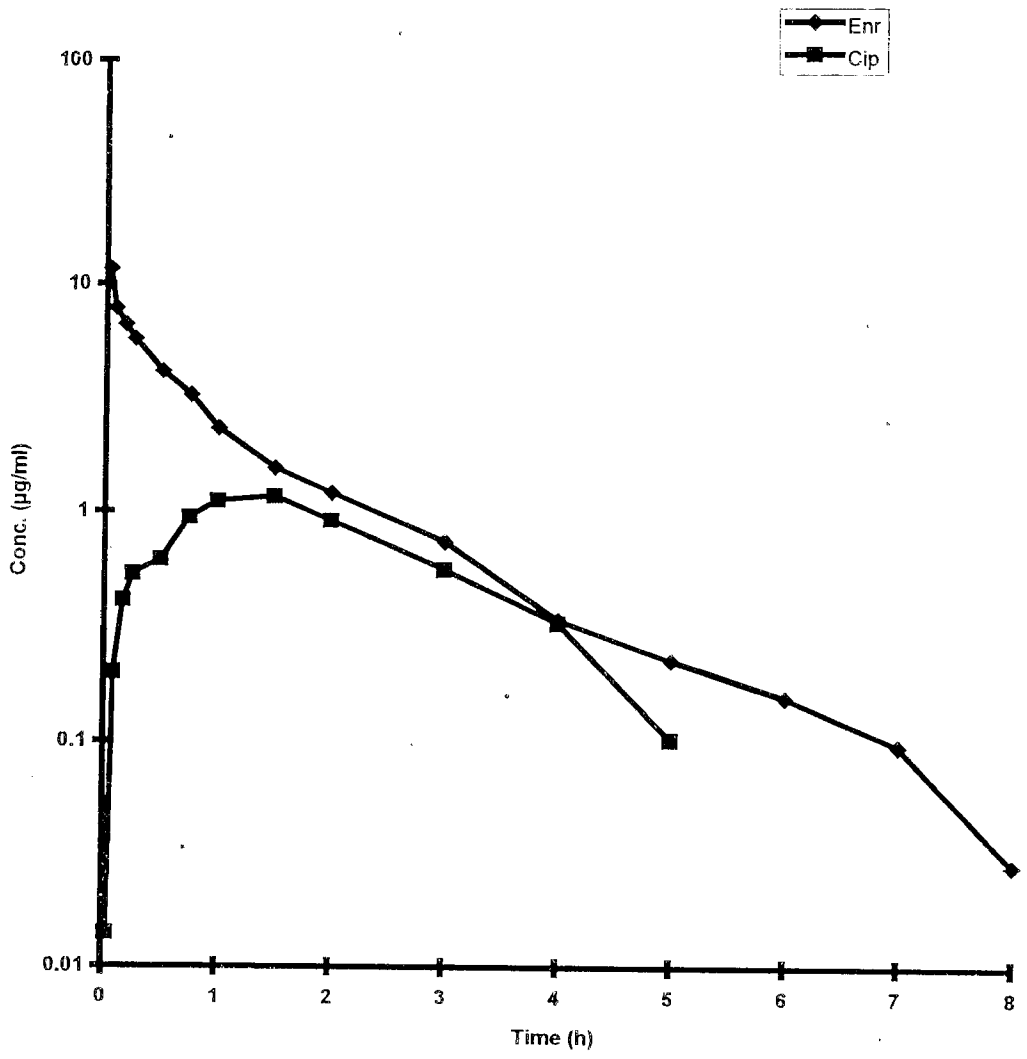

Fig. 5 Semilogarithmic plot of enrofloxacin and ciprofloxacin concentration in plasma vs. time following single intravenous administration of enrofloxacin (5 $mg.kg^{-1}$) in goats

The plasma concentration-time data of ENR could be best fitted to a two-compartment open model, as described by the equation;

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where Cp is the plasma concentration of ENR at time 't', A and B are zero-time intercepts of the biphasic curve and α and β are the first order rate constants of distribution and elimination phases, respectively, and 'e' is the base of natural logarithm.

Various pharmacokinetic parameters determined from plasma concentrations of ENR after its intravenous administration are summarised in Table. 8 The distribution (α) and elimination (β) phases and their zero time intercepts A and B of animal No 4, which is considered to be the representative of the group, are shown in Fig. 6.

The distribution rate constant of ENR varied from 2.649 to 61.95 h^{-1} with a median value of 6.217 h^{-1}. The median distribution half life was 0.115 h. The median value of elimination rate constant was 0.599 h^{-1} (range : 0.482-0.901 h^{-1}) and the median elimination half-life was 1.157 h. The median values of AUC, MRT, $V_{d\,area}$ and Cl_B of ENR were 9.95 $\mu g\,h.ml^{-1}$, 1.359 h, 0.863 $L.kg^{-1}$ and 502.5 $ml.h^{-1}.kg^{-1}$, respectively.

The plasma concentration-time data of CIP could be best fitted to a one-compartment open model, as described by the equation.

$$Cp = Be^{-\beta t} - A' e^{-kft}$$

where A' is the zero time intercept and k_f is the first order metabolite formation rate constant. Various pharmacokinetic parameters of ciprofloxacin are presented in Table 9. The median metabolite formation rate constant (k_f) observed

was 1.04 h^{-1} with a metabolite formation half-life ($t_{1/2\,kf}$) of 0.667 h. The metabolite ratio (MR) of CIP, as calculated by the ratio of AUC_{CIP}/AUC_{ENR} was 0.308 (0.196-0.624).

4.2 Plasma levels and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1})

The plasma concentrations of ENR and CIP at various time intervals after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) are presented in Tables 10 and 11, respectively. The graphical representation of mean plasma concentrations of ENR and CIP are depicted in Fig. 7.

Enrofloxacin was rapidly absorbed after subcutaneous administration with a mean plasma level of 0.295 ± 0.194 μg.ml^{-1} at 2 min. The peak plasma concentration (C_{max}) of 2.814 μg.ml^{-1}(1.403-3.88 μg.ml^{-1}) occurred at 1.0 h (t_{max}). Detectable concentrations were found upto 10 h.

CIP could be detected in the plasma from 10 minutes onwards, with a (C_{max} of 0.709 μg.ml^{-1} (0.342 - 0.978 μg.ml^{-1}) attained at 1.5 h (1.0-3.0 h). CIP was detected in the plasma upto 7 h.

The plasma concentrations, of both ENR and CIP could be fitted to a one-compartment open model, with first order absorption/metabolite formation as described by the equations,

$$C_p = Be^{-\beta t} - A' e^{-kat} \qquad \text{(enrofloxacin)}$$

$$C_p = Be^{-\beta t} - A' e^{-kft} \qquad \text{(ciprofloxacin)}$$

The pharmacokinetic parameters of ENR after a single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) are presented in Table 12 and the

Table 8 . Pharmacokinetics of enrofloxacin after single intravenous administration of enrofloxacin (5 mg.kg^{-1}) in normal goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
Cp^{o}	$\mu g.ml^{-1}$	9.148	7.723	14.251	21.39	59.40	14.25	(7.22-59.4)
A	$\mu g.ml^{-1}$	6.755	5.039	9.556	16.46	50.65	9.556	(5.04-50.6)
B	$\mu g.ml^{-1}$	2.393	2.684	4.695	4.93	8.75	4.695	(2.393-8.75)
α	h^{-1}	2.813	2.649	6.217	19.14	61.95	6.217	(2.65-61.95)
β	h^{-1}	0.482	0.792	0.599	0.544	0.901	0.599	(0.482-0.901)
$t_{1/2\,\alpha}$	h	0.246	0.262	0.115	0.036	0.011	0.115	(0.011-0.262)
$t_{1/2\,\beta}$	h	1.438	0.875	1.157	1.274	0.769	1.157	(0.769-1.438)
AUC	$\mu g.h.ml^{-1}$	7.59	5.45	9.95	10.66	11.31	9.95	(5.452-11.31)
AUMC	$\mu gh^{2}ml^{-1}$	12.26	5.05	13.53	21.05	13.51	13.51	(5.05-21.05)
MRT	h	1.615	0.927	1.359	1.975	1.194	1.359	(0.927-1.974)
$V_{d\,area}$	$L.\,kg^{-1}$	1.365	1.158	0.839	0.863	0.490	0.863	(0.49-1.365)
CI_B	$ml.\,h^{-1}\,kg^{-1}$	658.49	917.3	502.53	469.14	442.16	502.5	(442.2-917.3)
$V_{d\,ss}$	$L.\,kg^{-1}$	1.064	0.850	0.683	0.926	0.528	0.85	(0.528-1.064)
K_{12}	h^{-1}	0.998	0.587	2.934	12.848	47.705	2.934	(0.59-47.71)
K_{21}	h^{-1}	1.092	1.437	2.450	4.830	9.894	2.45	(1.09-9.89)
K_{el}	h^{-1}	1.205	1.417	1.432	2.006	5.252	1.432	(1.205-5.252)
V_c	$L.kg^{-1}$	0.546	0.647	0.351	0.234	0.084	0.351	(0.084-0.647)
V_p	$L.kg^{-1}$	0.499	0.264	0.420	0.622	0.405	0.42	(0.264-0.622)
Fc		0.4	0.559	0.418	0.272	0.171	0.4	(0.171-0.559)
T/P	ratio	1.5	0.789	1.391	2.687	4.829	1.5	(0.789-4.829)
K_{12}/K_{21}	ratio	0.914	0.408	1.197	2.660	4.822	1.197	(0.408-4.822)

Table 9. **Pharmacokinetics of ciprofloxacin after single intravenous administration of enrofloxacin (5 mg.kg^{-1}) in normal goats**

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
K_f	h^{-1}	1.015	2.389	0.720	1.04	1.18	1.04	(0.72-2.389)
β	h^{-1}	0.644	1.77	0.522	0.851	0.92	0.851	(0.522-1.77)
$t_{1/2\,Kf}$	h	0.683	0.290	0.963	0.667	0.589	0.667	(0.29-0.963)
$t_{1/2\,\beta}$	h	1.076	0.392	1.330	0.815	0.753	0.815	(0.392-1.33)
AUC	$\mu g.h.ml^{-1}$	4.74	1.68	5.47	2.09	2.38	2.38	(1.68-5.47)
AUMC	$\mu g.h^2 ml^{-1}$	11.28	1.65	14.39	4.22	4.47	4.47	(1.65-14.39)
MRT	h	2.38	0.985	2.63	2.02	1.87	2.02	(0.985-2.630)
MR	ratio	0.624	0.308	0.549	0.196	0.210	0.308	(0.19-0.62)
C_{max}	$\mu g.ml^{-1}$	1.576	1.270	2.13	0.818	1.201	1.27	(0.818-2.13)
t_{max}	h	1.0	0.75	2.00	1.5	1.0	1.0	(0.75-2.0)

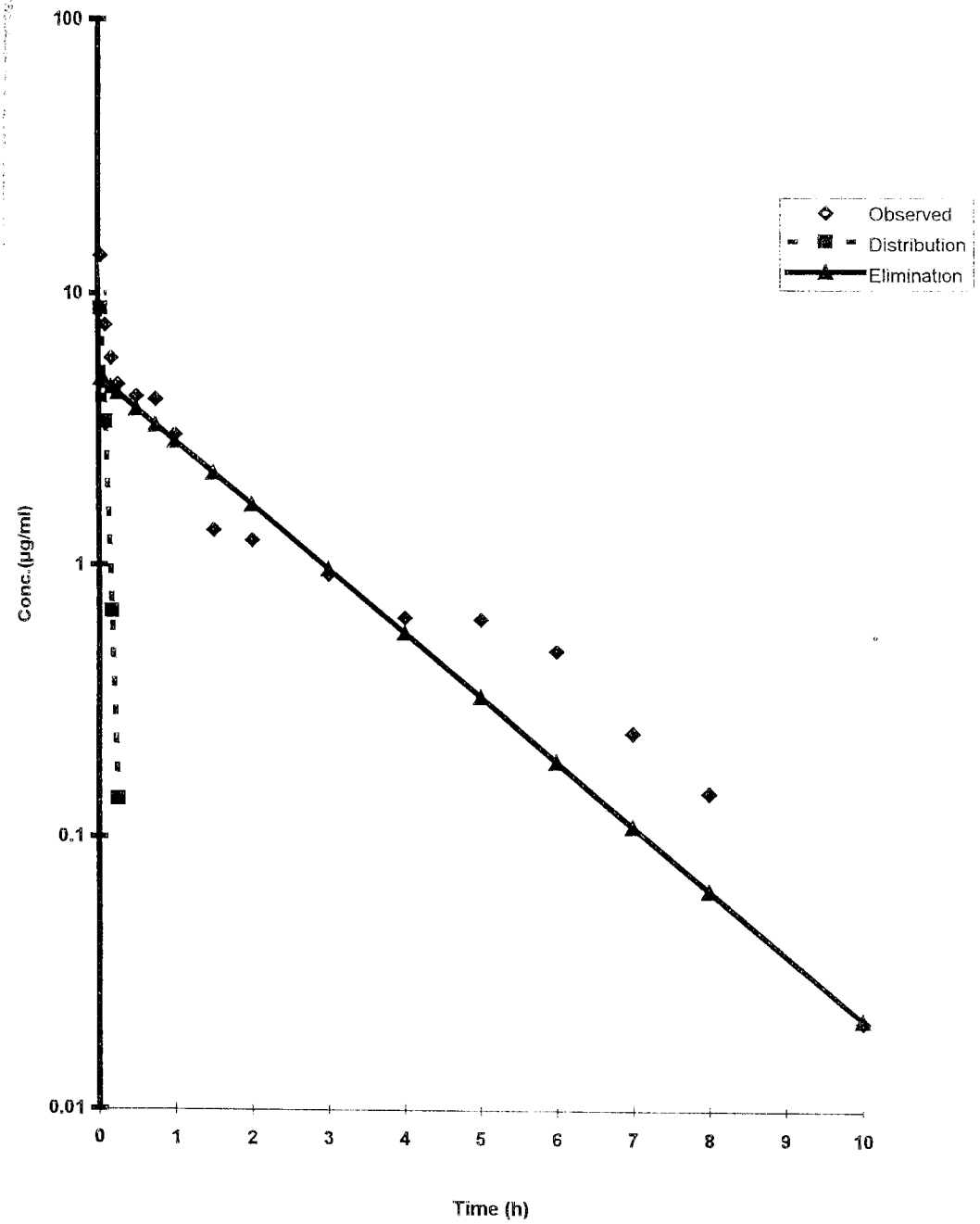

Fig. 6 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following single intravenous administration of enrofloxacin (5 mg.kg^{-1}) in goat no.4

Table 10. Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	–	0.131	0.177	0.105	1.061	0.295 ± 0.194
0.083	0.375	0.418	1.286	0.548	1.121	0.750 ± 0.189
0.167	0.774	0.618	1.926	1.232	1.883	1.287 ± 0.272
0.25	0.880	1.512	2.256	1.554	2.665	1.773 ± 0.312
0.5	0.953	1.994	2.806	2.239	3.702	2.339 ± 0.454
0.75	1.202	2.072	2.857	2.546	3.880	2.511 ± 0.442
1.0	1.324	1.940	2.979	2.819	3.646	2.542 ± 0.408
1.5	1.403	1.705	3.100	2.062	2.902	2.234 ± 0.331
2	1.347	1.290	2.647	1.509	2.730	1.905 ± 0.322
3	1.191	0.729	1.997	0.923	1.467	1.261 ± 0.222
4	0.939	0.568	1.572	0.270	1.148	0.899 ± 0.226
5	0.478	0.274	1.041	0.224	0.736	0.551 ± 0.152
6	0.421	0.115	0.809	0.187	0.432	0.393 ± 0.121
7	0.152	–	0.252	0.080	0.371	0.166 ± 0.066
8	–	–	0.105	–	0.120	0.045 ± 0.027
10	–	–	–	–	0.082	0.016 ± 0.016

Table 11. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal goats

Time (h)	1	2	3	4	5	Mean ± SE
0.167	-	0.044	0.059	-	-	0.020 ± 0.013
0.25	0.020	0.104	0.070	-	0.053	0.049 ± 0.018
0.5	0.047	0.109	0.202	0.073	0.083	0.103 ± 0.027
0.75	0.129	0.235	0.302	0.105	0.093	0.172 ± 0.041
1.0	0.471	0.342	0.701	0.162	0.167	0.370 ± 0.103
1.5	0.978	0.241	0.418	0.330	0.270	0.447 ± 0.136
2	0.625	0.278	0.238	0.782	0.402	0.465 ± 0.104
3	0.314	0.222	0.229	0.532	0.574	0.374 ± 0.075
4	0.184	0.162	0.149	0.230	0.303	0.206 ± 0.028
5	0.041	0.136	0.131	0.191	0.226	0.145 ± 0.031
6	-	0.053	0.022	0.072	0.147	0.058 ± 0.02
7	-	-	-	0.031	0.047	0.016 ± 0.009

Table 11. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033						
0.083						
0.167		0.044	0.059			0.020 ± 0.013
0.25	0.020	0.104	0.070		0.053	0.049 ± 0.018
0.5	0.047	0.109	0.202	0.073	0.083	0.103 ± 0.027
0.75	0.129	0.235	0.302	0.105	0.093	0.172 ± 0.041
1.0	0.471	0.342	0.701	0.162	0.167	0.370 ± 0.103
1.5	0.978	0.241	0.418	0.330	0.270	0.447 ± 0.136
2	0.625	0.278	0.238	0.782	0.402	0.465 ± 0.104
3	0.314	0.222	0.229	0.532	0.574	0.374 ± 0.075
4	0.184	0.162	0.149	0.230	0.303	0.206 ± 0.028
5	0.041	0.136	0.131	0.191	0.226	0.145 ± 0.031
6		0.053	0.022	0.072	0.147	0.058 ± 0.02
7				0.031	0.047	0.016 ± 0.009

Table 12. Pharmacokinetics of enrofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in normal goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
A'	μg.ml^{-1}	12.029	4.445	5.290	6.492	6.474	6.474	(4.445-12.029)
B	μg.ml^{-1}	12.330	4.444	5.289	6.492	6.474	6.474	(4.444-12.33)
Ka	h^{-1}	0.717	2.176	2.926	2.194	3.081	2.194	(0.719-3.081)
β	h^{-1}	0.533	0.576	0.384	0.696	0.476	0.533	(0.384-0.696)
$t_{1/2\,Ka}$	h	0.966	0.318	0.237	0.316	0.225	0.316	(0.225-0.966)
$t_{1/2\,\beta}$	h	1.301	1.203	1.805	0.996	1.456	1.301	(0.996-1.805)
AUC	μg.h.ml^{-1}	6.38	5.73	12.69	6.58	11.80	6.58	(5.73-12.69)
AUMC	μg.h^2.ml^{-1}	19.28	12.37	35.85	13.05	28.68	19.28	(12.37-35.85)
MRT	h	3.021	2.160	2.825	1.984	2.43	2.43	(1.984-3.021)
$V_{d\,area}$	L. kg^{-1}	1.24	1.59	1.31	0.674	0.928	1.24	(0.674-1.52)
Cl_B	ml. h^{-1} kg^{-1}	783.69	872.60	394.0	759.87	423.7	759.9	(394.0-872.6)
F	%	84.05	105.1	127.5	61.726	104.3	104.3	(61.73-127.5)
C_{max}	μg.ml^{-1}	1.403	2.072	3.10	2.819	3.88	2.819	(1.403-3.88)
t_{max}	h	1.5	0.75	1.5	1.00	0.75	1.0	(0.75-1.5)
MAT	h	1.406	1.233	1.466	0.009	1.237	1.237	(0.009-1.466)

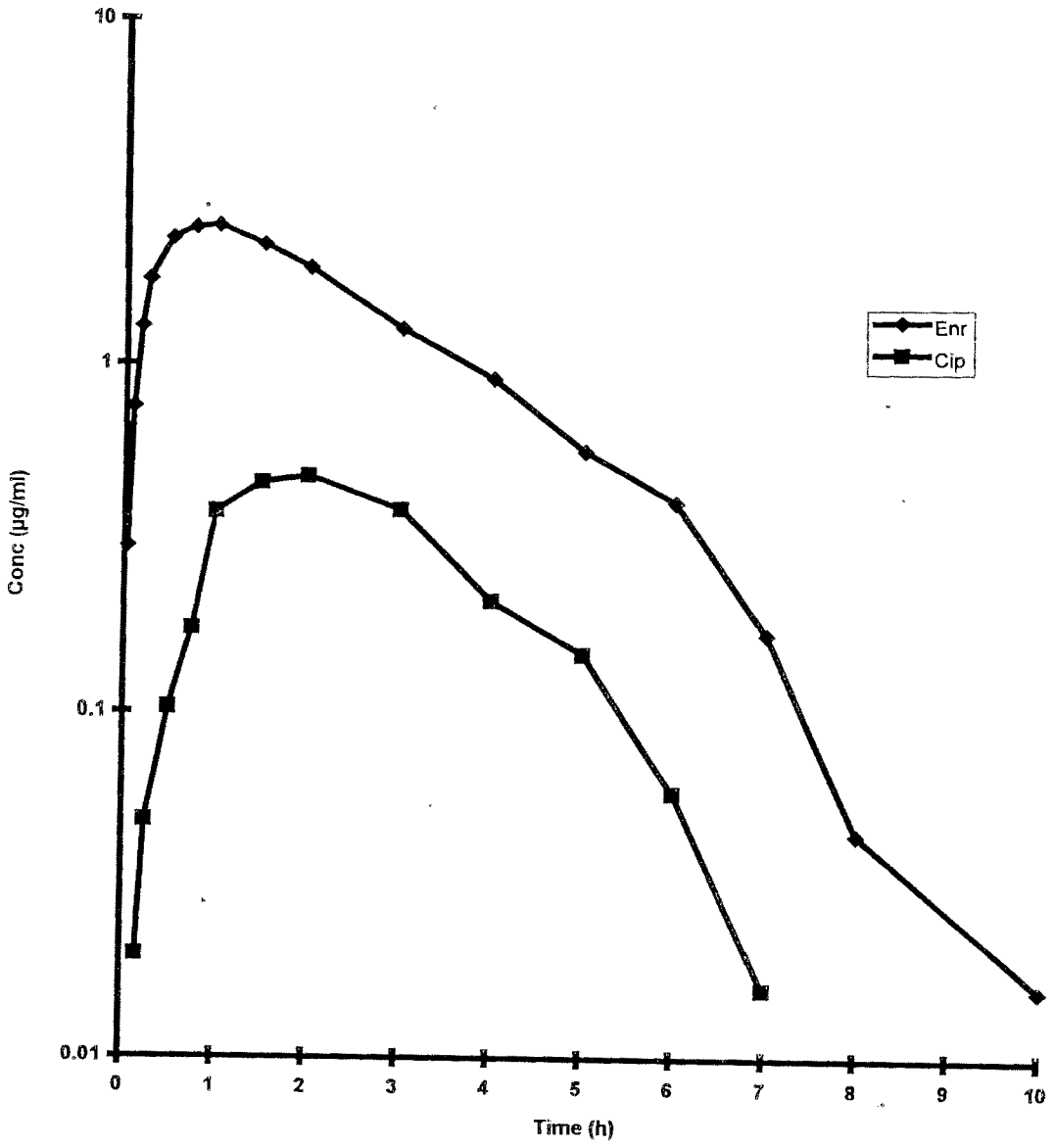

Fig. 7 Semilogarithmic plot of enrofloxacin and ciprofloxacin concentrations in plasma vs. time following single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal goats

regression lines indicating absorption (ka) and elimination (β) phases for goat No. 4, considered to be a representative of the group, are shown in Fig. 8.

The absorption half-life of ENR varied from 0.225 to 0.966 h with a median value of 0.316 h and the median elimination half life ($t_{1/2\beta}$) was 1.301 h (0.996-1.805 h). The median (range) values of AUC, AUMC and MRT were 6.58 (5.73-12.69) $\mu g.h.ml^{-1}$, 19.28 (12.37- 35.85) $\mu g.h^2.ml^{-1}$ and 2.43 (1.984-3.021) h, respectively. Based on the ratio of AUC of subcutaneous route to that of intravenous route (AUC s.c./AUC i.v.), the bioavailability of ENR was found to be 104.3 (61.73-127.5) %.

The pharmacokinetic parameters of CIP are presented in Table 13. The median metabolite formation half-life was found to be 0.827 h and the median elimination half-life was 1.259 (1.096 - 1.638) h . The metabolite ratio (MR) ranged between 0.11-0.296 with a median value 0.192.

4.3 Plasma concentrations and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in febrile goats

Escherichia coli endotoxin induced marked pyrexia in goats. A significant elevation of rectal temperature was recorded at 1 h after the administration of endotoxin. An increase in rectal temperature of 1-1.5°F was maintained up to 12 h after endotoxin administration (Table 14).

The plasma concentrations of ENR and CIP in febrile goats are presented in Tables 15 and 16, respectively and in Fig. 9. The absorption (k_a) and elimination (β) phases and their zero-time intercepts A' and B, of goat No 2, considered to be the representative of the group, are shown in Fig. 10.

Enrofloxacin could be detected in the plasma right from 2 minutes after

Table 13. Pharmacokinetics of ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in normal goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
K_f	h^{-1}	0.837	1.001	1.36	0.789	0.547	0.837	(0.547-1.136)
β	h^{-1}	0.625	0.447	0.632	0.55	0.423	0.55	(0.423-0.632)
$t_{1/2kf}$	h	0.827	0.692	0.610	0.878	1.266	0.827	(0.61-1.266)
$t_{1/2\beta}$	h	1.109	1.550	1.096	1.259	1.638	1.259	(1.096-1.638)
AUC	$\mu g.h.ml^{-1}$	1.77	1.10	1.39	1.95	1.94	1.77	(1.10-1.95)
AUMC	μgh^2ml^{-1}	4.09	3.39	3.45	6.01	7.05	4.09	(3.39-7.05)
MRT	h	2.315	3.07	2.487	3.09	3.642	3.07	(2.315-3.642)
MR	ratio	0.227	0.192	0.110	0.296	0.164	0.192	(0.11-0.296)
C_{max}	$\mu g.ml^{-1}$	0.978	0.342	0.709	0.782	0.574	0.709	(0.342-0.978)
t_{max}	h	1.5	1.00	1.0	2.0	3.00	1.5	(1.00-3.00)

Table 14. Effect of *E coli* endotoxin on rectal temperature in goats

Time after administration (h)	Rectal temperature (°F)
0	102.4 ± 0.433
1	103.7 ± 0.195
2	104.4 ± 0.117
3	104.6 ± 0.063
4	104.4 ± 0.365
5	104.4 ± 0.547
6	104.6 ± 0.329
7	104.4 ± 0.167
8	104.8 ± 0.343
9	104.7 ± 0.372
10	104.1 ± 0.388
11	103.8 ± 0.395
12	103.6 ± 0.444

Table 15. Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in febrile goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	0.059	0.216	0.116	0.510	0.176	0.215 ± 0.078
0.083	0.242	0.330	0.199	0.481	0.462	0.343 ± 0.056
0.167	0.349	0.582	0.489	0.868	0.911	0.640 ± 0.109
0.25	1.096	0.909	1.021	2.509	0.950	1.297 ± 0.305
0.5	1.951	1.364	1.591	3.044	2.854	2.161 ± 0.336
0.75	2.272	2.431	2.577	2.973	2.509	2.552 ± 0.117
1.0	2.449	2.076	1.791	2.789	2.679	2.357 ± 0.187
1.5	2.506	1.935	3.118	2.432	2.993	2.597 ± 0.212
2	1.440	1.699	2.822	1.876	2.327	2.033 ± 0.245
3	1.016	1.497	1.572	0.750	1.313	1.230 ± 0.153
4	0.815	1.122	1.422	0.556	0.861	0.955 ± 0.147
5	0.442	0.785	0.857	0.429	0.734	0.649 ± 0.089
6	0.175	0.617	0.403	0.274	0.434	0.381 ± 0.075
7	0.108	0.483	0.266	0.171	0.296	0.265 ± 0.064
8	–	0.372	0.373	0.142	0.171	0.212 ± 0.072
10	–	0.223	0.128	0.043	–	0.078 ± 0 043
12	–	0.130	0.011	–	–	0.028 ± 0.025

Table 16. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after single subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in febrile goats

Time (h)	1	2	3	4	5	Mean ± SE
0.083	0.027	-	-	-	-	0.005 ± 0.005
0.167	0.057	-	-	-	-	0.011 ± 0.011
0.25	0.122	-	-	-	-	0.024 ± 0.024
0.5	0.114	0.015	-	0.025	-	0.031 ± 0.021
0.75	0.156	0.085	0.05	0.052	0.011	0.071 ± 0.024
1.0	0.343	0.172	0.112	0.119	0.038	0.157 ± 0.051
1.5	0.517	0.223	0.172	0.272	0.091	0.255 ± 0.072
2	0.658	0.254	0.213	0.359	0.156	0.328 ± 0.089
3	0.442	0.412	0.475	0.418	0.232	0.396 ± 0.042
4	0.313	0.442	0.668	0.319	0.085	0.365 ± 0.095
5	0.180	0.357	0.360	0.288	0.066	0.250 ± 0.056
6	0.023	0.289	0.138	0.191	0.022	0.133 ± 0.051
7	-	0.121	0.085	0.095	-	0.060 ± 0.025
8	-	0.079	0.022	0.035	-	0.027 ± 0.015

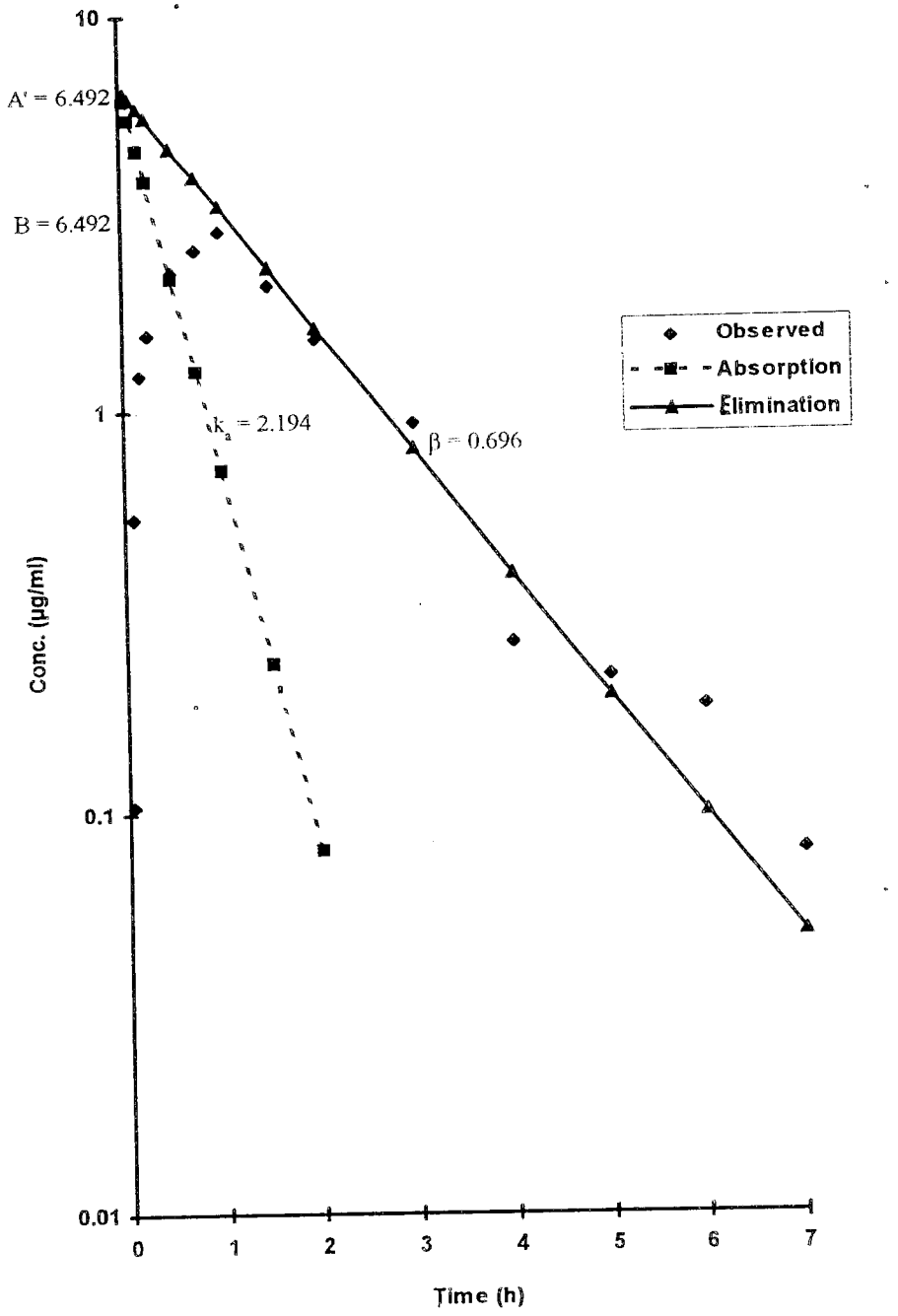

Fig. 8 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following single subcutaneous administration of enrofloxacin (5 mg.kg^{-1})in goat no.4

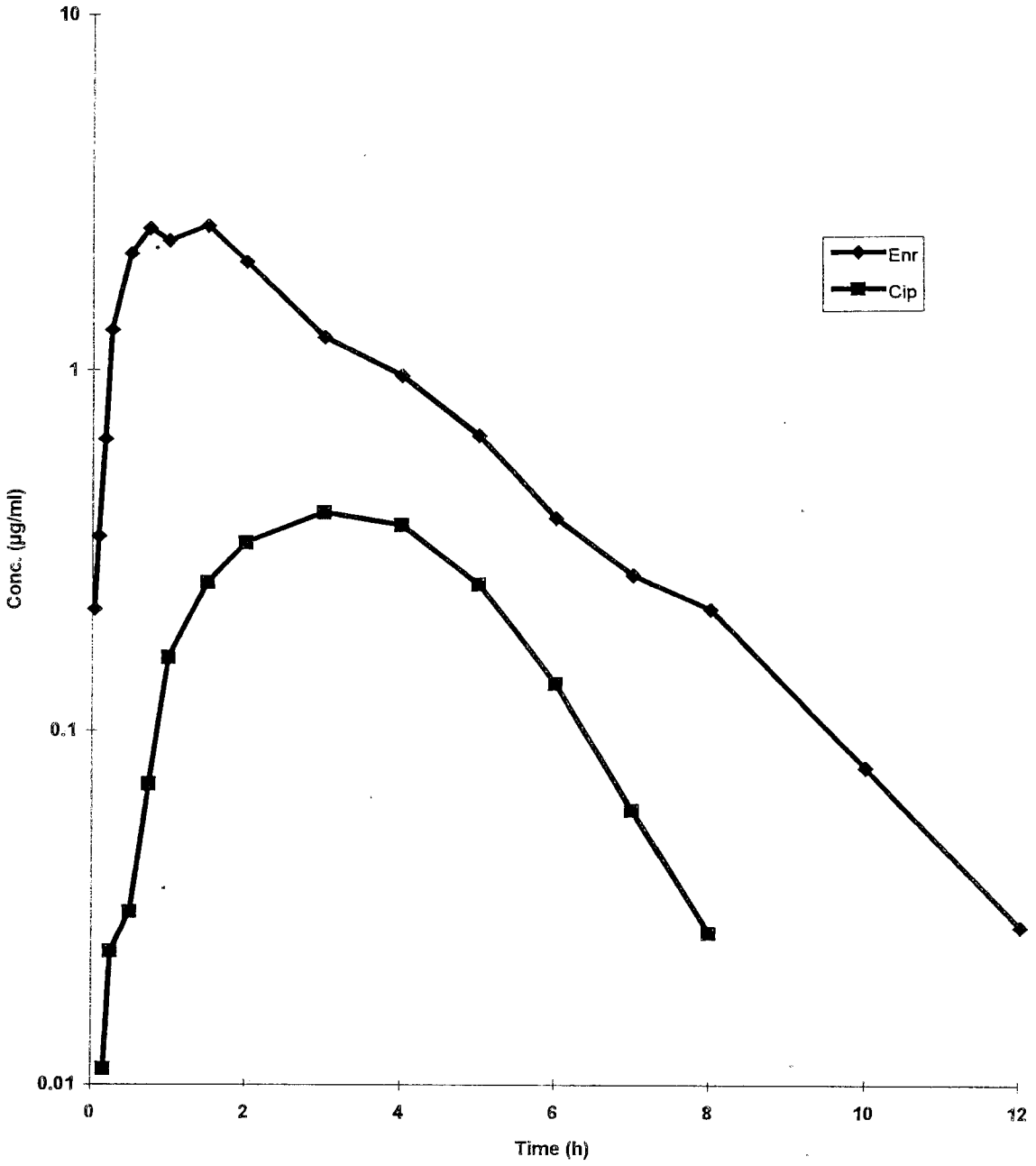

Fig. 9 Semilogarithmic plot of enrofloxacin and ciprofloxacin concentrations in plasma following subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in febrile goats

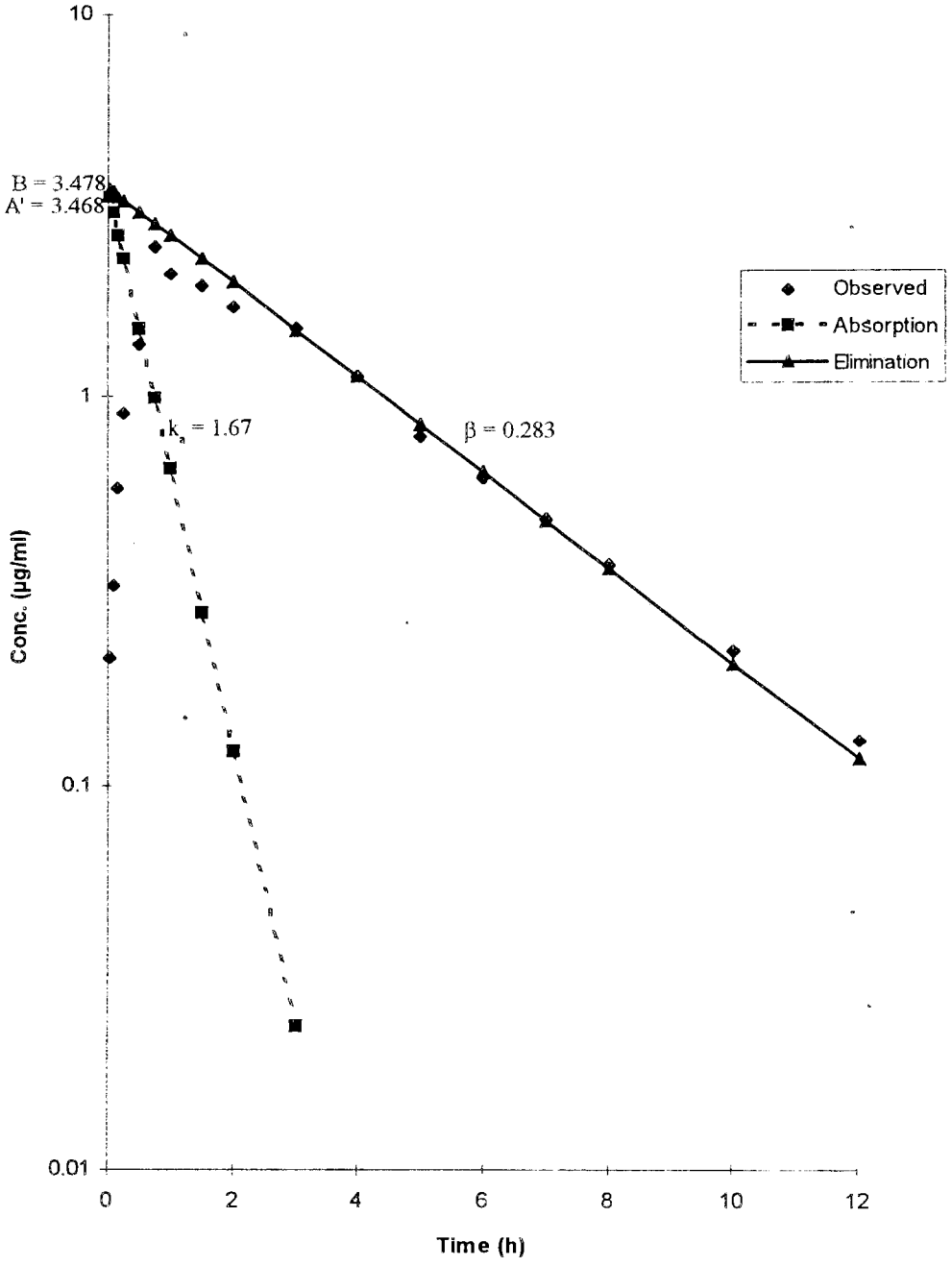

Fig. 10 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in febrile goat no.2

administration with mean plasma level of 0.215 ± 0.078 μg.ml^{-1}. The peak plasma concentration (C_{max}) of 2.993 (2.431-3.118) μg.ml^{-1} was achieved at (t_{max}) 1.5 (0.75-1.50) h. Detectable concentration of enrofloxacin could be found upto 12 h. Ciprofloxacin could be detected in the plasma from 10 minutes. The peak plasma concentration (C_{max}) of 0.442 (0.232-0.668) μg.ml^{-1} was observed at 3.0 h.

There were no significant differences in the concentrations of both ENR and CIP between normal and febrile goats (Table 17). However, consistently lower concentrations of enrofloxacin were observed in febrile animals up to 0.5 h as compared to the normal goats (Fig. 11). In the later plasma samples (from 1.5 to 10 h), there were higher levels of enrofloxacin in febrile goats, with the detectable concentrations observed upto 12 h as compared to only 10 h in normal goats. A similar pattern of lower concentrations of CIP in the earlier plasma samples (upto 2 h) was observed. Similar to ENR, the CIP concentrations persisted in the plasma up to 8 h in febrile goats as compared to 7 h in normal goats (Fig. 12).

Various pharmacokinetic parameters determined in febrile goats for both ENR and CIP are summarized in Tables 18 and 19 As in normal goats, the pharmacokinetics of enrofloxacin and ciprofloxacin could be described by a one compartment open model with first order absorption/metabolite formation. The median elimination half-life ($t_{1/2\,\beta}$) was 1.361 h with a range of 1.081-2.452 h. The AUC, MRT, $V_{d\,area}$ and Cl_B of ENR in febrile goats were found to be 10.53 μg.h.ml^{-1}, 3.075 h, 1.03 L.kg^{-1} and 474.6 ml.h^{-1}.kg^{-1}. The mean absorption time (MAT) was 1.724 h.

The median elimination half-life of CIP, after single subcutaneous administration of ENR, was 1.222 h, and the median metabolite formation half-life ($t_{1/2\,kf}$) was 1.170 h. The metabolite ratio (MR) ranged from 0.072-0.275 with a median of 0.188.

Table 17. Effect of endotoxin-induced fever on the plasma concentrations of enrofloxacin and ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in five goats

Time after drug admin (h)	Enrofloxacin		Ciprofloxacin	
	Normal	Febrile	Normal	Febrile
0.033	0.295±0.194	0.215±0.078	-	-
0.083	0.750±0.189	0.343±0.056	-	-
0.167	1.287±0.272	0.640±0.109	0.020±0.013	0.011±0.011
0.25	1.773±0.312	1.297±0.305	0.049±0.018	0.024±0.024
0.5	2.339±0.454	2.161±0.336	0.103±0.027	0.031±0.021
0.75	2.511±0.442	2.552±0.117	0.172±0.041	0.071±0.024
1.0	2.542±0.408	2.357±0.187	0.370±0.103	0.157±0.051
1.5	2.234±0.331	2.597±0.212	0.447±0.136	0.255±0.072
2.0	1.905±0.322	2.033±0.245	0.465±0.104	0.328±0.089
3.0	1.261±0.222	1.230±0.153	0.374±0.075	0.396±0.042
4.0	0.899±0.226	0.955±0.147	0.206±0.028	0.365±0.095
5.0	0.551±0.152	0.649±0.089	0.145±0.031	0.250±0.056
6.0	0.393±0.121	0.381±0.075	0.059±0.020	0.133±0.051
7.0	0.166±0.066	0.265±0.064	0.016±0.009	0.060±0.025
8.0	0.045±0.027	0.212±0.072	-	0.027±0.015
10	0.016±0.016	0.078±0.043	-	-
12	-	0.028±0.025	-	-

Values are expressed as µg of enrofloxacin/ciprofloxacin/ml of plasma and represent the Mean ± SE

Table 18. Pharmacokinetics of enrofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in febrile goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
A'	µg/ml^{-1}	9.721	3.468	18.749	8.336	5.405	8.336	(3.468-18.749)
B	µg/ml^{-1}	9.711	3.478	18.748	8.401	5.405	8.401	(3.478-18.748)
K_a	h^{-1}	1.189	1.67	0.804	1.306	1.747	1.306	(0.804-1.747)
β	h^{-1}	0.641	0.283	0.553	0.509	0.404	0.509	(0.283-0.641)
$t_{1/2ka}$	h	0.583	0.415	0.862	0.531	0.397	0.531	(0.397-0.862)
$t_{1/2\beta}$	h	1.081	2.452	1.254	1.361	1.717	1.361	(1.081-2.452)
AUC	µg.h.ml^{-1}	7.13	10.30	11.03	10.57	10.53	10.53	(7.13-11.03)
AUMC	µgh^{2}ml^{-1}	17.00	43.07	34.00	30.31	32.60	32.6	(17.0-43.07)
MRT	h	2.382	4.182	3.083	2.867	3.075	3.075	(2.382-4.182)
$V_{d\,area}$	L. kg^{-1}	1.03	3.24	0.908	0.929	1.09	1.03	(0.908-3.24)
Cl_B	ml. h^{-1} kg^{-1}	700.8	485.5	453.4	473.0	474.6	474.6	(453.4-700.8)
F	%	93.9	188.9	110.7	99.15	93.10	99.15	(93.1-188.9)
C_{max}	µg.ml^{-1}	2.506	2.431	3 118	3.044	2.993	2.993	(2.431-3.118)
t_{max}	h	1.5	0.75	1.5	0.75	1.5	1.5	(0.75-1.5)
MAT	h	0.767	3.255	1.724	0.892	1.901	1.724	(0.767-3.255)

Table 19. Pharmacokinetics of ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in febrile goats

		Goat Number						
Parameter	Unit	1	2	3	4	5	Median	(range)
K_f	h^{-1}	0.697	0.504	0.567	0.592	0.650	0.592	(0.504-0.697)
β	h^{-1}	0.606	0.358	0.508	0.422	0.625	0.568	(0.358-0.625)
$t_{1/2kf}$	h	0.994	1.375	1.222	1.170	1.066	1.170	(0.994-1.375)
$t_{1/2\beta}$	h	1.144	1.936	1.222	1.643	1.108	1.222	(1.108-1.936)
AUC	$\mu g.h.ml^{-1}$	1.96	2.27	2.07	1.88	0.76	1.96	(0.76-2.27)
AUMC	μgh^2ml^{-1}	5.39	10.70	8.32	7.58	2.61	7.58	(2.61-10 70)
MRT	h	2.75	4.71	4.01	4.037	3.45	4.01	(2.75-4.71)
MR	ratio	0.275	0.220	0.188	0.178	0.072	0.188	(0.072-0.275)
C_{max}	$\mu g.ml^{-1}$	0.658	0.442	0.668	0.418	0.232	0.442	(0.232-0.668)
t_{max}	h	2.0	4.0	4.0	3.0	3.0	3.0	(2.0-4.0)

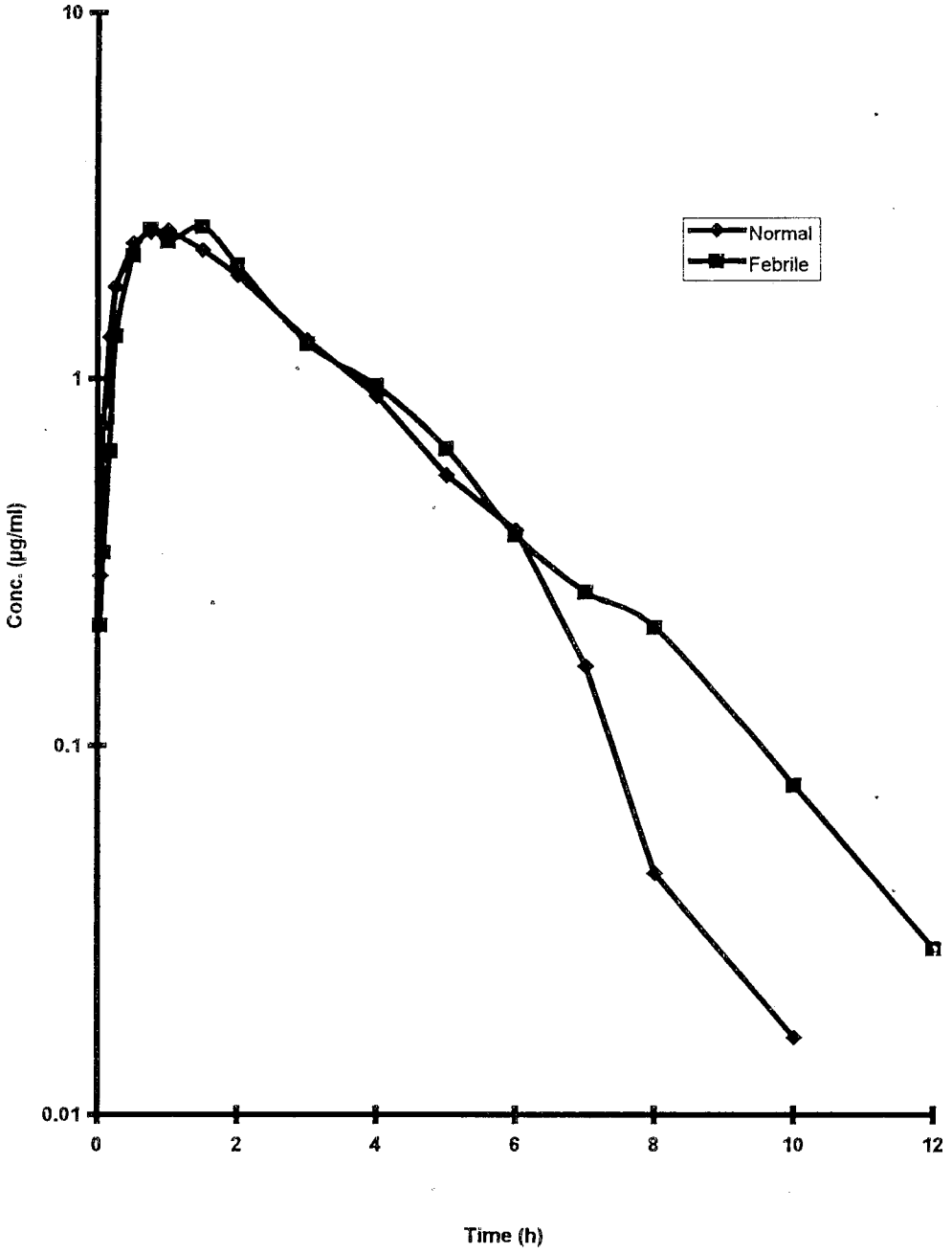

Fig-11 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in normal and febrile goats

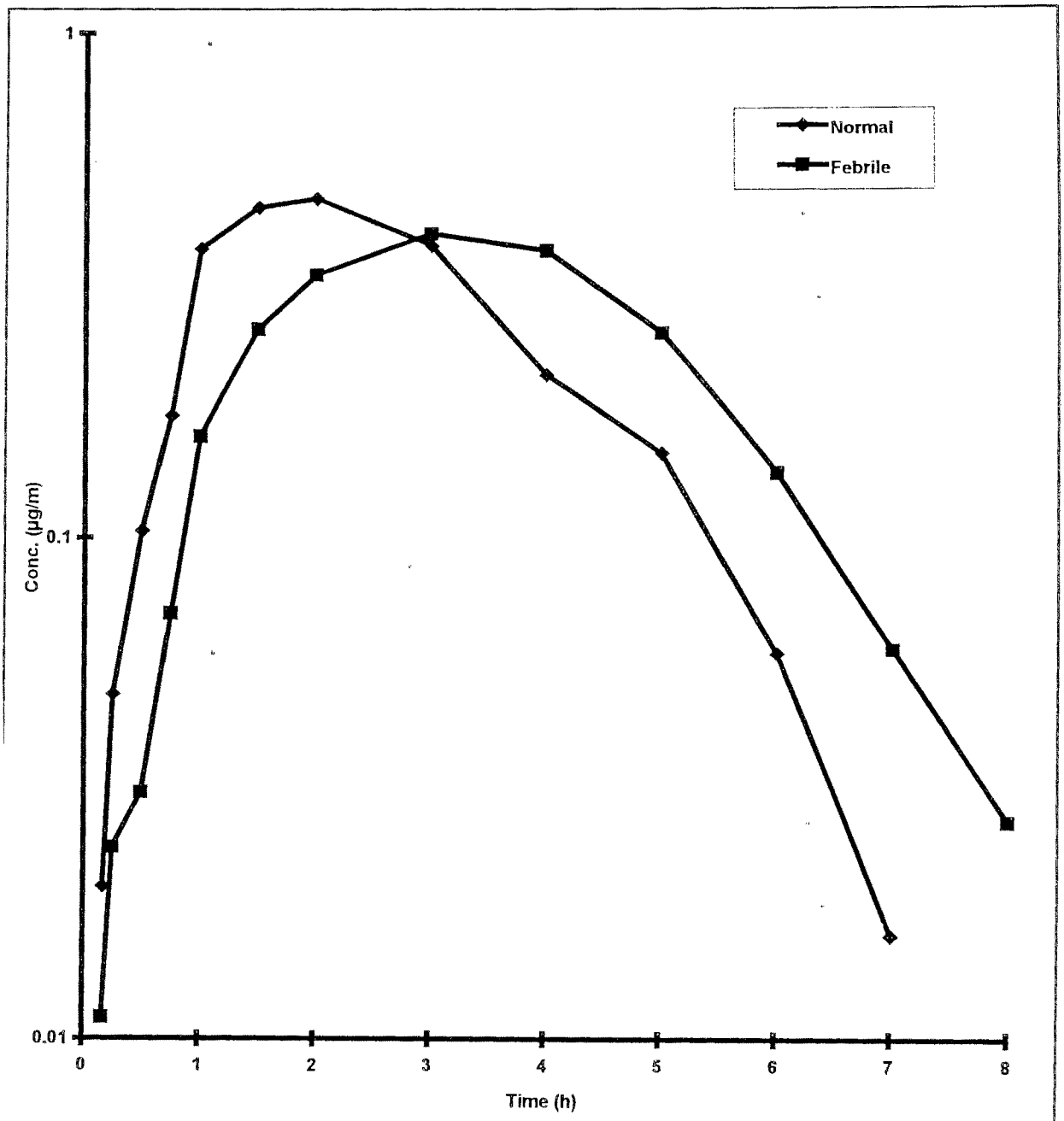

Fig. 12 Semilogarithmic plot of ciprofloxacin concentration in plasma vs. time following subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in normal and febrile goats

There were no significant differences between the pharmacokinetic parameters of both ENR and CIP in febrile goats, when compared with those obtained in normal goats (Table 20). However, apparently higher vlaues of AUC, MRT and MAT of ENR were observed in febrile goats as compared to normal goats. On the contrary, much lower value of Cl_B was determined in febrile than in normal goats.

On ciprofloxacin pharmacokinetics, appreciably lower value of C_{max} was observed in febrile animals, while the t_{max} was considerably prolonged to 3 h as compared to 1.5 h in normal goats.

4.4 Plasma concentrations and pharmacokinetics of enrofloxacin and ciprofloxacin after coadministration of piperine (2 mg.kg^{-1}, s.c.) and enrofloxacin (5 mg.kg^{-1}, s.c.)

The plasma levels of ENR and CIP at various time intervals after concurrent administration of piperine and ENR are presented in Table 21 and 22, respectively. A graphical representation of the mean plasma levels is also presented in Fig. 13. The absorption (k_a) and elimination phases (β), along with the zero-time intercepts (A' and B) for goat No. 1, are shown in Fig. 14.

On administration of piperine (2 mg.kg^{-1} s.c.) in dimethyl sulphoxide as a 1.25% solution, irritation at the site of injection was observed as evidenced by discomfort and pain displayed by the animals soon after administration. This however, was transient and the animals were normal within a few minutes time of injection. Mild hemolysis was observed in the earlier plasma samples collected up to 4 h from piperine-treated animals.

ENR could be detected in the plasma from 2 minutes. Detectable concentrations of enrofloxacin were found upto 12 h in 4 animals while in one animal the drug was detected upto 24 h. The peak plasma concentration of

Table 20. Effect of endotoxin-induced fever on the pharmacokinetics of enrofloxacin and ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in goats

		ENROFLOXACIN				CIPROFLOXACIN			
Parameter	Unit	Normal		Febrile		Normal		Febrile	
A'	$\mu g.ml^{-1}$	**6.474**	(4.445-12.029)	**8.336**	(3.468-18.749)	–	–	–	–
B	$\mu g.ml^{-1}$	**6.474**	(4.444-12.330)	**8.401**	(3.478-18.748)	–	–	–	–
K_a	h^{-1}	**2.194**	(0.717-3.081)	**1.306**	(0.804-1.747)	–	–	–	–
K_f	h^{-1}	–	–	–	–	**0.837**	(0.547-1.136)	**0.592**	(0.504-0.697)
β	h^{-1}	**0.533**	(0.384-0.696)	**0.509**	(0.283-0.641)	**0.550**	(0.423-0.632)	**0.568**	(0.358-0.625)
$t_{1/2\,ka}$	h	**0.316**	(0.225-0.966)	**0.531**	(0.397-0.862)	–	–	–	–
$t_{1/2\,kf}$	h	-	-	-	-	**0.827**	(0.61-1.266)	**1.170**	(0.994-1.375)
$t_{1/2\,\beta}$	h	**1.301**	(0.996-1.805)	**1.361**	(1.081-2.452)	**1.259**	(1.096-1.638)	**1.222**	(1.108-1.936)
AUC	$\mu g.h.ml^{-1}$	**6.58**	(5.73-12.69)	**10.53**	(7.13-11.03)	**1.77**	(1.10-1.95)	**1.96**	(0.76-2.27)
AUMC	$\mu g.h^2.ml^{-1}$	**19.28**	(12.37-35.85)	**32.6**	(17.0-43.07)	**4.09**	(3.39-7.05)	**7.58**	(2.61-10.70)
MRT	h	**2.43**	(1.984-3.021)	**3.075**	(2.382-4.182)	**3.07**	(2.315-3.642)	**4.01**	(2.75-4.71)
$V_{d\,area}$	$L.kg^{-1}$	**1.24**	(0.674-1.52)	**1.03**	(0.908-3.24)	–	–	–	–
Cl_B	$ml.h^{-1}\,kg^{-1}$	**759.87**	(394.0-872.6)	**474.6**	(453.4-700.8)	–	–	–	–
F	%	**104.3**	(61.73-127.5)	**99.15**	(93.1-188.9)	–	–	–	–
MR	ratio	-	-	-	-	**0.192**	(0.110-0.296)	**0.188**	(0.072-0.275)
C_{max}	$\mu g\,ml^{-1}$	**2.819**	(1.403-3.88)	**2.993**	(2.431-3.118)	**0.709**	(0.342-0.978)	**0.442**	(0.232-0.668)
t_{max}	h	**1.0**	(0.75-1.5)	**1.5**	(0.75-1.5)	**1.5**	(1.0-3.0)	**3.0**	(2.0-4.0)
MAT	h	**1.237**	(0.009-1.466)	**1.724**	(0.767-3.255)	-	-	-	–

Values are expressed as **Median** (range) of five animals

Table 21. Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin after concurrent administration of enrofloxacin (5 $mg.kg^{-1}$,s.c.) and piperine (2 $mg.kg^{-1}$, s.c.) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	0.089	0.158	0.087	-	0.037	0.074 ± 0.027
0.083	0.114	0.284	0.103	0.850	0.387	0.348 ± 0.136
0.167	0.265	0.550	0.350	0.909	0.618	0.538 ± 0.113
0.25	0.348	0.868	0.646	1.159	0.785	0.801 ± 0.141
0.5	0.373	1.041	0.734	1.686	1.342	1.035 ± 0.228
0.75	0.446	1.436	0.780	1.642	1.726	1.206 ± 0.252
1.0	0.423	1.628	0.835	1.942	2.029	1.371 ± 0.317
1.5	0.651	1.732	0.964	2.107	3.022	1.695 ± 0.422
2	0.947	1.809	1.247	1.673	2.458	1.627 ± 0.258
3	0.951	1.768	1.375	2.019	1.234	1.569 ± 0.186
4	1.057	1.718	1.509	3.033	1.458	1.755 ± 0.337
5	1.022	1.649	1.608	1.320	1.518	1.423 ± 0.116
6	0.869	1.253	1.408	1.143	1.221	1.178 ± 0.089
7	0.677	1.033	1.134	0.924	0.942	0.942 ± 0.076
8	0.539	0.670	0.777	0.914	0.751	0.730 ± 0.062
10	0.360	0.402	0.434	0.583	0.421	0.44 ± 0.038
12	0.292	0.050	0.284	0.313	0.200	0.228 ± 0.11
24	-	-	-	0.054	-	0.011 ± 0.011

Table 22. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after concurrent administration of enrofloxacin (5 $mg.kg^{-1}$,s.c.) and piperine (2 $mg.kg^{-1}$, s.c.) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.167	0.023	-	0.052	-	-	0.015 ± 0.010
0.25	0.043	0.010	0.068	0.015	-	0.027 ± 0.012
0.5	0.067	0.068	0.111	0.044	0.011	0.060 ± 0.016
0.75	0.133	0.105	0.128	0.118	0.060	0.109 ± 0.013
1.0	0.113	0.087	0.188	0.336	0.137	0.172 ± 0.044
1.5	0.177	0.119	0.231	0.837	0.138	0.300 ± 0.136
2	0.157	0.158	0.242	0.618	0.140	0.263 ± 0.091
3	0.282	0.217	0.286	0.359	0.137	0.256 ± 0.037
4	0.295	1.44	0.41	0.136	0.235	0.303 ± 0.056
5	0.303	0.289	0.334	0.098	0.218	0.248 ± 0.042
6	0.694	0.250	0.313	0.060	0.191	0.302 ± 0.107
7	0.710	0.222	0.286	0.05	0.162	0.286 ± 0.113
8	0.58	0.130	0.241	0.04	0.116	0.221 ± 0.095
10	0.493	0.10	0.215	-	0.074	0.176 ± 0.086
12	0.319	-	0.127	-	0.062	0.102 ± 0.059
24	0.030	-	0.074	-	0.049	0.031 ± 0.041

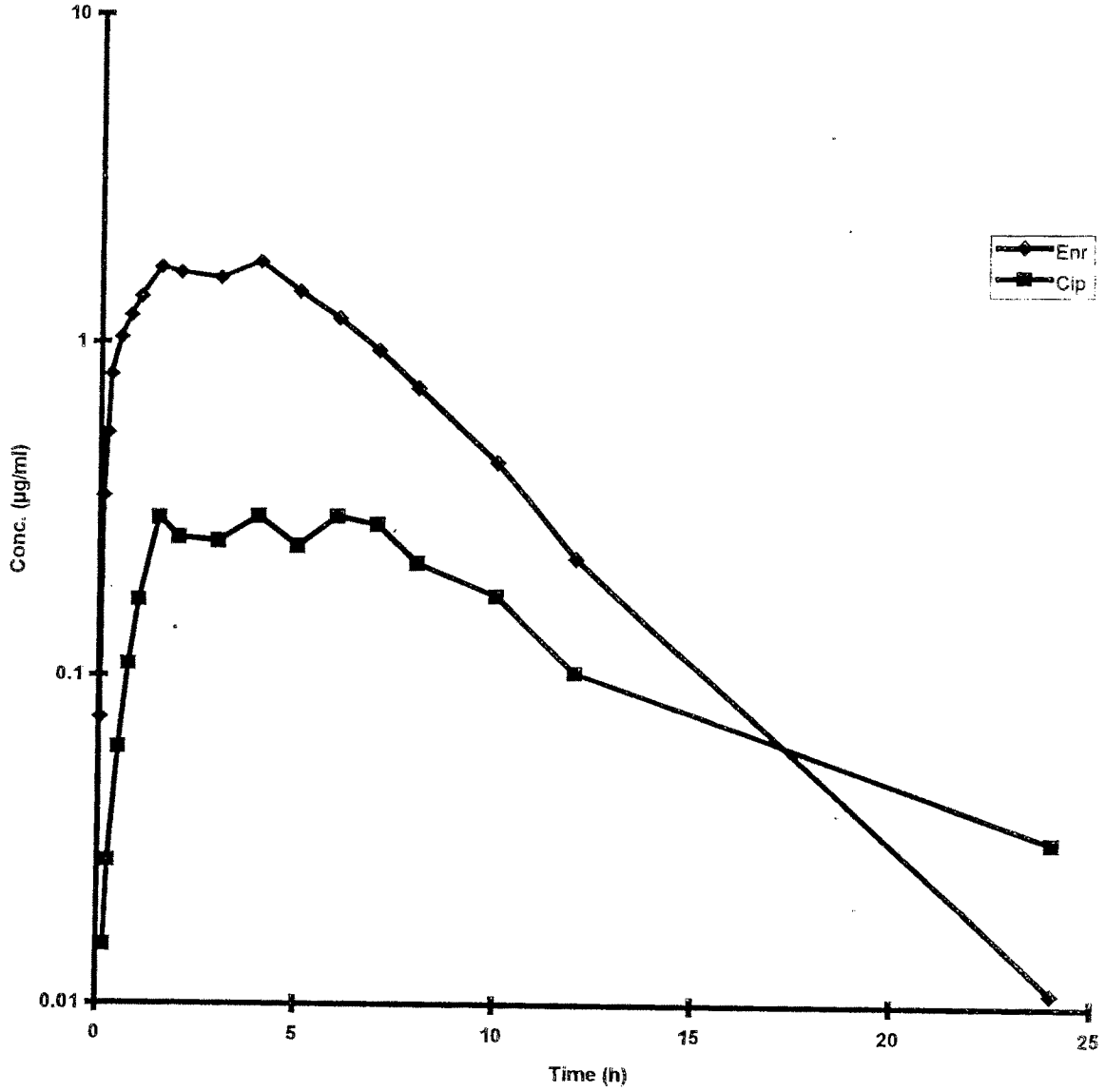

Fig. 13 Semilogarithmic plot of enrofloxacin and ciprofloxacin concentrations in plasma vs. time following concurrent administration of enrofloxacin (5 $mg.kg^{-1}$, s.c.) and piperine (2 $mg.kg^{-1}$, s.c.) in goats

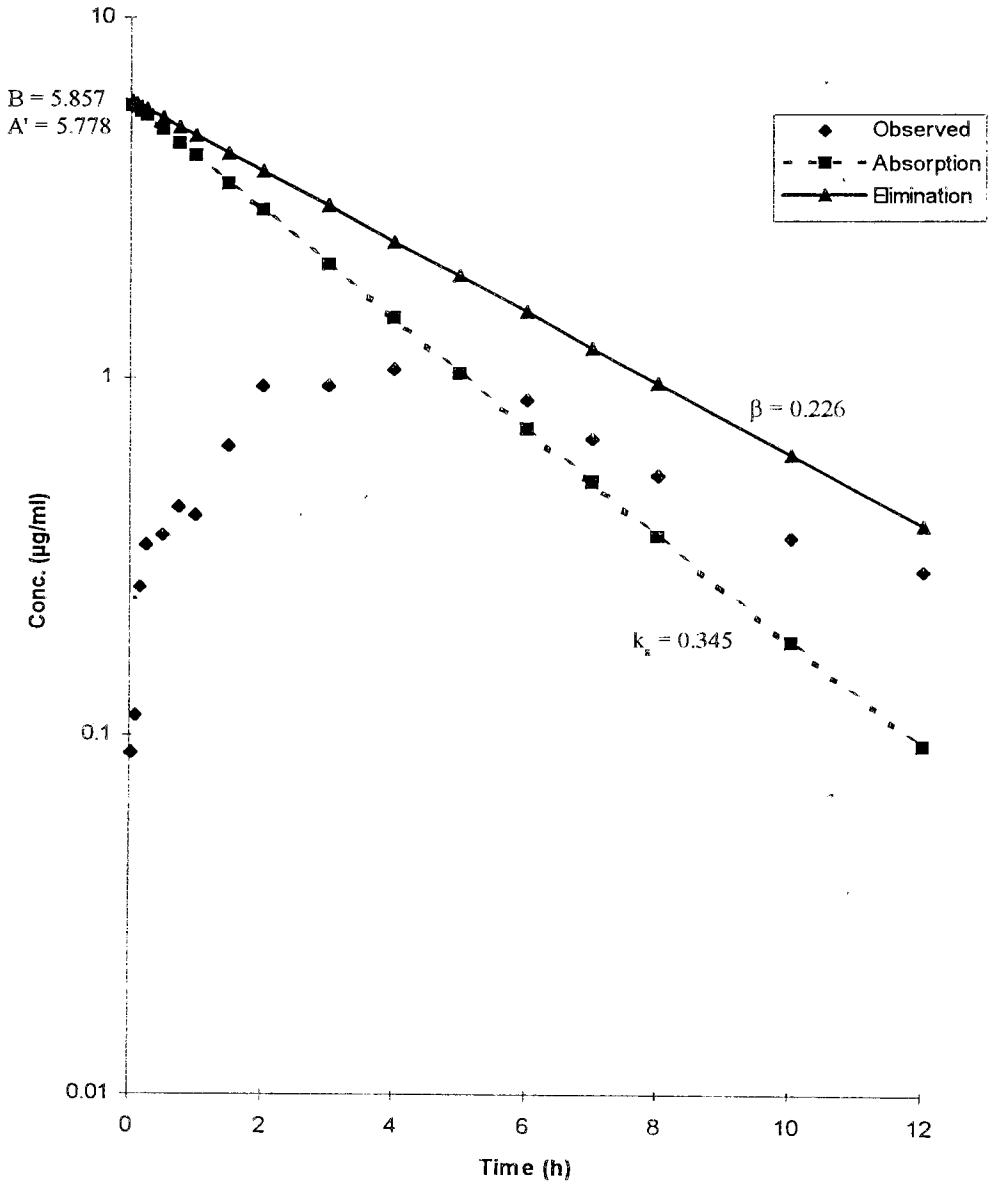

Fig. 14 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following concurrent administration of enrofloxacin (5 mg.kg^{-1}, s.c.) and piperine (2 mg.kg^{-1}, s.c.) in goat no. 1

enrofloxacin (C_{max}) was 1.809 μg.ml^{-1} with a range of 1.057-3.033 μg.ml^{-1}. The median t_{max} was 4.0 h with a range of 1.5 - 5.0 h.

CIP could be detected in the plasma from 10 min after administration of ENR in two animals, from 15 minutes in another two animals and from 30 minutes in the fifth animal. The C_{max} observed was 0.440 (0.235-0.837) μg.ml^{-1}. The median t_{max} was 4.0 (range 1.5 - 7.0) h.

The pharmacokinetics of ENR and CIP could be described by a one compartment open model. The detailed pharmacokinetic parameters calculated for goats in phase IV of the study are presented in Tables 24 and 25, respectively.

The absorption half-life of ENR ranged from 0.542 to 2.008 h with a median of 1.452 h and the elimination half-life ranged from 1.883 to 3.154 h with a median of 3.012 h. The AUC ranged between 9.18 and 17.95 μg.h.ml^{-1} with a median of 12.71 μg.h.ml^{-1}. The median values of $V_{d\,area}$, Cl_B and F were 2.13 L.kg^{-1}, 393.5 ml.h^{-1}. kg^{-1} and 132.9%, respectively.

The median pharmacokinetic values of CIP in goats after concurrent administration of ENR (5 mg.kg^{-1}, s.c.) and piperine (2 mg.kg^{-1}, s.c.) were, $t_{1/2\beta}$, 2.961 h, $t_{1/2\,kf}$, 2.242 h and metabolite ratio, 0.243.

The plasma levels and pharmacokinetic variables of ENR and CIP obtained in goats in phase IV of the study were compared with those of normal animals (Tables 23 and 26).

ENR plasma concentrations were significantly lower from 15 minutes to 1 h. Whereas, these were significantly higher from 5 to 10 h in piperine-treated group as compared to normal goats. While ENR could not be detected after 10 h in normal animals, appreciable concentrations of ENR was measured upto 12 h and even upto 24 h in one animal (Fig. 15).

Table 23. Effect of piperine (2 mg.kg^{-1}, s.c.) on the plasma concentrations of enrofloxacin and ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in five goats

Time after drug admin (h)	Enrofloxacin		Ciprofloxacin	
	Normal	piperine-treated	Normal	piperine-treated
0.033	0.295±0.194	0.074±0.027	-	-
0.083	0.750±0.189	0.348±0.136	-	-
0.167	1.287±0.272	0.538±0.113	0.020±0.013	0.015±0.010
0.25	1.773±0.312	0.801±0.141*	0.049±0.018	0.027±0.012
0.5	2.339±0.454	1.035±0.228*	0.103±0.027	0.060±0.016
0.75	2.511±0.442	1.206±0.252*	0.172±0.041	0.109±0.013
1.0	2.542±0.408	1.371±0.317*	0.370±0.103	0.172±0.044
1.5	2.234±0.331	1.695±0.422	0.447±0.136	0.300±0.136
2.0	1.905±0.322	1.627±0.258	0.465±0.104	0.263±0.091
3.0	1.261±0.222	1.569±0.186	0.374±0.075	0.256±0.037
4.0	0.899±0.226	1.755±0.337	0.206±0.028	0.303±0.056
5.0	0.551±0.152	1.423±0.116**	0.145±0.031	0.248±0.042
6.0	0.393±0.121	1.178±0.089**	0.059±0.020	0.302±0.107
7.0	0.166±0.066	0.942±0.076**	0.016±0.009	0.286±0.113
8.0	0.045±0.027	0.730±0.062**	-	0.221±0.095
10	0.016±0.016	0.440±0.038**	-	0.176±0.086
12	-	0.228±0.110	-	0.102±0.059
24	-	0.011±0.011	-	0.031±0.041

[1]Values are expressed as µg of enrofloxacin/ciprofloxacin/ml of plasma and represent the Mean ± SE

* Significantly different ($P<0.05$) from respective normal values

** Significantly different ($P<0.01$) from respective normal values

Table 24. Pharmacokinetics of enrofloxacin after concurrent administration of enrofloxacin (5 mg.kg^{-1}, s.c.) and piperine (2 mg.kg^{-1}, s.c.) in goats

		Goat Number						
Parameter	Unit	1	2	3	4	5	Median	(range)
A^1	µg.ml^{-1}	5.778	17.674	10.035	4.234	4.248	5.778	(4.234-17.674)
B	µg.ml^{-1}	5.857	17.839	10.091	4.967	4.248	5.857	(4.248-17.839)
k_a	h^{-1}	0.345	0.477	0.383	0.684	1.28	0.477	(0.345-1.28)
β	h^{-1}	0.226	0.368	0.258	0.22	0.23	0.23	(0.220-0.368)
$t_{1/2\,ka}$	h	2.008	1.452	1.809	1.014	0.542	1.452	(0.542-2.008)
$t_{1/2\beta}$	h	3.066	1.883	2.685	3.154	3.012	3.012	(1.883-3.154)
AUC	µg.h.ml^{-1}	9.18	12.43	12.71	17.95	15.04	12.71	(9.18-17.95)
AUMC	µgh^{2}ml^{-1}	62.77	57.52	76.64	107.23	73.78	73.78	(57.52-107.23)
MRT	h	6.837	4.628	6.03	5.973	4.91	5.973	(4.628-6.837)
$V_{d\,area}$	L. kg^{-1}	2.91	2.49	1.95	2.13	1.92	2.13	(1.92-2.91)
Cl_B	ml. h^{-1} kg^{-1}	544.6	402.3	393.5	278.55	332.5	393.5	(278.5-544.6)
F	%	120.9	228.0	127.7	168.4	132.9	132.9	(120.9-228.0)
C_{max}	µg.ml^{-1}	1.057	1.809	1.608	3.033	3.022	1.809	(1.057-3.033)
t_{max}	h	4.0	2.0	5.0	4.0	1.5	4.0	(1.5-5.0)
MAT	h	5.222	3.701	4.671	3.998	3.716	3.998	(3.701-5.222)

Table 25. Pharmacokinetics of ciprofloxacin after concurrent administration of enrofloxacin (5 $mg.kg^{-1}$, s.c.) and piperine (2 $mg.kg^{-1}$, s.c.) in goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
K_f	h^{-1}	0.196	0.307	0.309	0.787	0.783	0.309	(0.196-0.787)
β	h^{-1}	0.142	0.234	0.245	0.516	0.119	0.234	(0.119-0.516)
$t_{1/2kf}$	h	3.536	2.257	2.242	0.881	0.886	2.242	(0.881-3.536)
$t_{1/2\beta}$	h	4.885	2.961	2.828	1.342	5.836	2.961	(1.342-5.836)
AUC	$\mu g.h.ml^{-1}$	7.11	3.40	3.09	1.85	2.65	3.09	(1.85-7.11)
AUMC	μgh^2ml^{-1}	68.71	20.00	20.16	5.46	33.61	20.16	(5.46-68.71)
MRT	h	9.66	5.88	6.52	2.947	12.69	6.52	(2.947-12.69)
MR	ratio	0.775	0.274	0.243	0.103	0.176	0.243	(0.103-0.775)
C_{max}	$\mu g.ml^{-1}$	0.710	0.440	0.410	0.837	0.235	0.440	(0.235-0.837)
t_{max}	h	7.0	4.0	4.0	1.5	4.0	4.0	(1.5-7.0)

Table 26. Effect of piperine on the pharmacokinetics of enrofloxacin and ciprofloxacin after concurrent administration of enrofloxacin (5 mg.kg^{-1},s.c.) and piperine (2 mg.kg^{-1}, s.c.) in goats

Parameter	Unit	ENROFLOXACIN				CIPROFLOXACIN			
		Normal		Piperine-treated		Normal		Piperine-treated	
A'	μg ml^{-1}	6.474	(4.445-12.029)	5.778	(4.234-17.674)	–	–	–	–
B	μg ml^{-1}	6.474	(4.444-12.330)	5.857	(4.248-17.839)	–	–	–	–
K_a	h^{-1}	2.194	(0.717-3.081)	0.477*	(0.345-1.280)	–	–	–	–
K_f		–	–	–	–	0.837	(0.547-1.136)	0.309	(0.196-0.787)
β	h^{-1}	0.533	(0.384-0.696)	0.230*	(0.220-0.368)	0.550	(0.423-0.632)	0.234*	(0.119-0.516)
$t_{1/2\,ka}$	h	0.316	(0.225-0.966)	1.452*	(0.542-2.008)	–	–	–	–
$t_{1/2\,kf}$	h	–	–	–	–	0.827	(0.61-1.266)	2.242	(0.881-3.536)
$t_{1/2\,\beta}$	h	1.301	(0.996-1.805)	3.012*	(1.883-3.154)	1.259	(1.096-1.638)	2.961*	(1.342-5.836)
AUC	μg.h.ml^{-1}	6.58	(5.73-12.69)	12.71*	(9.18-17.95)	1.77	(1.10-1.95)	3.09	(1.85-7.11)
AUMC	μg.h^2.ml^{-1}	19.28	(12.37-35.85)	73.78*	(57.52-107.23)	4.09	(3.39-7.05)	20.16	(5.46-68.71)
MRT	h	2.43	(1.984-3.021)	5.973*	(4.628-6.837)	3.07	(2.315-3.642)	6.52	(2.947-12.69)
$V_{d\,area}$	L. kg^{-1}	1.24	(0.674-1.52)	2.13*	(1.92-2.91)	–	–	–	–
Cl_B	ml. h^{-1} kg^{-1}	759.87	(394-872.6)	393.5*	(278.5-544.6)	–	–	–	–
F	%	104.3	(61.73-127.5)	132.9*	(120.9-228.0)	–	–	–	–
MR	ratio	–	–	–	–	0.192	(0.110-0.296)	0.243	(0.103-0.775)
C_{max}	μg.ml^{-1}	2.819	(1.403-3.88)	1.809*	(1.057-3.033)	0.709	(0.342-0.978)	0.440	(0.235-.837)
t_{max}	h	1.0	(0.75-1.5)	4.0*	(1.5-5.0)	1.5	(1.0-3.0)	4.0	(1.5-7.0)
MAT	h	1.237	(0.009-1.466)	3.998*	(3.701-5.222)	–	–	–	–

Values are expressed as **Median** (range) of five animals

* Significantly different ($P<0.05$) from respective normal values

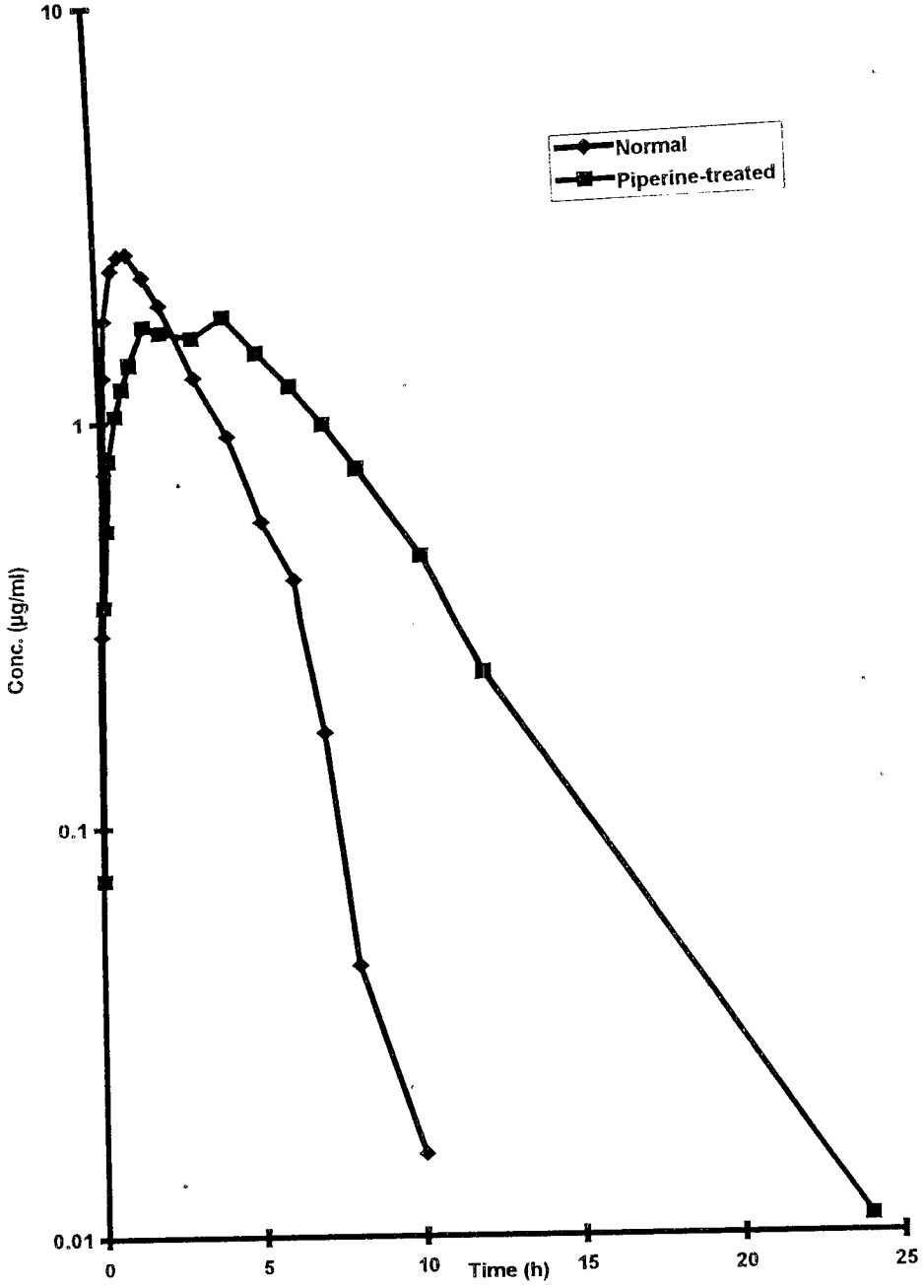

Fig- 15 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal and piperine-treated goats

As compared to normal animals, CIP plasma levels remained lower up to 3 h and thereafter these levels were higher and could be detected upto 24 h in piperine-treated animals (Fig. 16).

The values of all the important pharmacokinetic parameters such as $t_{1/2\,\beta}$, AUC, MRT, $V_{d\,area}$, F and MAT were significantly higher in piperine-treated animals as compared to normal goats and the Cl_{β} was significantly lower in piperine-treated goats. Piperine significantly increased $t_{1/2\,\beta}$ of CIP and there was an appreciable increase in the values of its AUC and MRT as compared to normal goats.

4.5 Plasma concentrations and pharmacokinetics of enrofloxacin and ciprofloxacin after co-administration of probenecid (40 mg.kg^{-1}, s.c.) and enrofloxacin (5 mg.kg^{-1}, s.c.)

The plasma concentrations of ENR and CIP in animals of phase V of the study are depicted in Fig 17. The absorption (k_a) and elimination phases (β), and their zero-time intercepts (A' and B) of goat No. 5, considered to be a representative of the group are shown in Fig. 18. The mean plasma concentrations of ENR and CIP are also presented in Table 27 and 28, respectively. The plasma concentrations of ENR were detected in the plasma right from 2 minutes to 12 h. The median peak plasma concentration (C_{max}) of ENR was 2.534 µg.ml^{-1} which was recorded at 3.0 h (t_{max}).

CIP could also be detected in the plasma right from two minutes and detectable concentrations were found upto 24 h. The median C_{max} of CIP was 0.315 µg.ml^{-1} observed at 3.0 h. The plasma concentration of both ENR and CIP were compared in probenecid-treated and normal goats and the results are presented in Table 29 and Fig. 19 and 20.

The pharmacokinetics of both ENR and CIP were described by a one-compartment open model. The important pharmacokinetic parameters of ENR and CIP in phase V of the study are presented in Table 30 and 31, respectively.

Table 27. Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin after concurrent administration of enrofloxacin (5 $mg.kg^{-1}$,s.c.) and probenecid (40 $mg.kg^{-1}$, s.c.) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	0.110	–	–	–	0.057	0.033 ± 0.022
0.083	0.720	0.047	0.037	0.123	0.220	0.229 ± 0.126
0.167	1.169	0.239	0.102	0.335	0.495	0.468 ± 0.187
0.25	1.688	0.549	0.208	0.737	0.622	0.761 ± 0.246
0.5	2.806	0.991	0.337	1.160	1.085	1.276 ± 0.409
0.75	2.882	1.377	0.839	1.465	1.723	1.657 ± 0.338
1.0	3.002	2.013	0.933	1.402	1.787	1.827 ± 0.346
1.5	3.654	2.314	1.287	2.270	2.533	2.412 ± 0.377
2	2.950	2.334	1.561	2.534	3.131	2.502 ± 0.275
3	1.864	2.359	2.077	2.410	4.426	2.627 ± 0.460
4	0.880	2.147	1.615	2.056	4.388	2.17 ± 0.587
5	0.518	1.682	1.884	1.785	2.910	1.756 ± 0.380
6	0.513	1.592	1.358	1.274	1.860	1.319 ± 0.225
7	0.227	1.221	1.238	0.698	1.251	0.927 ± 0.203
8	0.113	0.914	1.065	0.467	0.861	0.684 ± 0.173
10	0.052	0.536	0.693	0.171	0.441	0.332 ± 0.120
12	–	0.303	0.434	0.081	0.210	0.206 ± 0.077

Table 28. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after concurrent administration of enrofloxacin (5 $mg.kg^{-1}$,s.c.) and probenecid (40 $mg.kg^{-1}$, s.c.) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	0.036	–	–	–	0.022	0.012 ± 0.007
0.083	0.101	–	–	–	0.029	0.026 ± 0.019
0.167	0.110	–	–	–	0.080	0.038 ± 0.024
0.25	0.117	–	0.01	0.027	0.102	0.051 ± 0.024
0.5	0.139	0.024	0.026	0.055	0.136	0.076 ± 0.026
0.75	0.178	0.068	0.078	0.087	0.110	0.104 ± 0.020
1.0	0.325	0.108	0.021	0.089	0.096	0.128 ± 0.052
1.5	0.421	0.191	0.048	0.165	0.122	0.189 ± 0.063
2	0.400	0.213	0.056	0.285	0.175	0.226 ± 0.057
3	0.360	0.315	0.086	0.251	0.460	0.294 ± 0.062
4	0.294	0.293	0.113	0.269	0.368	0.267 ± 0.042
5	0.268	0.249	0.121	0.277	0.346	0.252 ± 0.037
6	0.212	0.272	0.231	0.309	0.246	0.254 ± 0.017
7	0.166	0.221	0.133	0.198	0.188	0.181 ± 0.015
8	0.104	0.196	0.121	0.136	0.120	0.135 ± 0.016
10	0.081	0.139	0.098	0.088	0.070	0.095 ± 0.012
12	0.023	0.090	0.087	0.073	0.035	0.062 ± 0.014
24	–	0.016	0.060	0.029	0.015	0.024 ± 0.010

Table 29. Effect of probenecid (40 mg.kg^{-1}, s.c.) on the plasma concentrations of enrofloxacin and ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg.kg^{-1}) in five goats

Time after drug admin (h)	Enrofloxacin		Ciprofloxacin	
	Normal	probenecid-treated	Normal	probenecid-treated
0.033	0.295±0.194	0.033±0.022	-	0.012±0.007
0.083	0.750±0.189	0.229±0.126	-	0.026±0.019
0.167	1.287±0.272	0.468±0.187	0.020±0.013	0.038±0.024
0.25	1.773±0.312	0.761±0.246	0.049±0.018	0.051±0.024
0.5	2.339±0.454	1.276±0.409	0.103±0.027	0.076±0.026
0.75	2.511±0.442	1.657±0.338	0.172±0.041	0.104±0.020
1.0	2.542±0.408	1.827±0.346	0.370±0.103	0.128±0.052
1.5	2.234±0.331	2.412±0.370	0.447±0.136	0.189±0.063*
2.0	1.905±0.322	2.502±0.275	0.465±0.104	0.226±0.057*
3.0	1.261±0.222	2.627±0.460*	0.374±0.075	0.294±0.062
4.0	0.899±0.226	2.217±0.587	0.206±0.028	0.267±0.042
5.0	0.551±0.152	1.756±0.380*	0.145±0.034	0.252±0.037*
6.0	0.393±0.121	1.319±0.225*	0.059±0.020	0.254±0.017**
7.0	0.166±0.066	0.927±0.203*	0.016±0.009	0.181±0.015**
8.0	0.045±0.027	0.684±0.173*	-	0.135±0.016
10	0.016±0.016	0.332±0.120*	-	0.095±0.012
12	-	0.206±0.077	-	0.062±0.014
24	-	-	-	0.024±0.010

Values are expressed as µg of enrofloxacin/ciprofloxacin/ml of plasma and represent the Mean ± SE

* Significantly different ($P<0.05$) from respective normal values

** Significantly different ($P<0.01$) from respective normal values

Table 30. Pharmacokinetics of enrofloxacin after concurrent administration of enrofloxacin (5 $mg.kg^{-1}$, s.c.) and probenecid (40 $mg.kg^{-1}$, s.c.) in goats

		Goat Number						
Parameter	Unit	1	2	3	4	5	Median	(range)
A'	$\mu g.ml^{-1}$	9.703	8.320	10.758	28.095	46.788	10.758	(8.32-46.788)
B	$\mu g.ml^{-1}$	9.702	8.319	10.757	28.095	46.788	10.757	(8.319-46.788)
k_a	h^{-1}	1.474	0.624	0.369	0.540	0.449	0.54	(0.369-1.474)
β	h^{-1}	0.550	0.272	0.238	0.429	0.375	0.375	(0.238-0.550)
$t_{1/2\,ka}$	h	0.470	1.112	1.876	1.284	1.543	1.284	(0.47-1.876)
$t_{1/2\beta}$	h	1.261	2.546	2.911	1.617	1.848	1.848	(1.269-2.911)
AUC	$\mu g.h.ml^{-1}$	11.34	17.26	15.91	13.91	23.14	15.91	(11.34-23.14)
AUMC	μgh^2ml^{-1}	28.66	91.23	103.89	56.10	102.76	91.23	(28.66-103.89)
MRT	h	2.526	5.285	6.528	4.032	4.440	4.44	(2.526-6.528)
$V_{d\,area}$	$L.\,kg^{-1}$	1.19	3.37	2.11	1.093	1.178	1.19	(1.093-3.37)
Cl_B	$ml.\,h^{-1}\,kg^{-1}$	440.8	289.6	314.16	359.44	216.06	314.16	(216.06-440.8)
F	%	149.4	316.7	159.9	130.50	204.5	159.9	(130.6-316.7)
C_{max}	$\mu g.ml^{-1}$	3.654	2.359	2.077	2.534	4.426	2.534	(2.077-4 426)
t_{max}	h	1 5	3.0	3.0	2.0	3.0	3.0	(1.5-3.0)
MAT	h	0.911	4.358	5.169	2.057	3 247	3.247	(0.911-5.169)

Table 31. Pharmacokinetics of ciprofloxacin after concurrent administration of enrofloxacin (5 mg.kg^{-1}, s.c.) and probenecid (40 mg.kg^{-1}, s.c.) in goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
K_f	h^{-1}	0.497	0.439	0.221	0.393	0.373	0.393	(0.221-0.497)
β	h^{-1}	0.348	0.187	0.129	0.209	0.244	0.209	(0.129-0.348)
$t_{1/2kf}$	h	1.395	1.579	3.148	1.764	1.857	1.764	(1.395-3.148)
$t_{1/2\beta}$	h	1.993	3.697	5.376	3.314	2.846	3.314	(1.993-5.376)
AUC	$\mu g.h.ml^{-1}$	2.46	3.06	1.59	2.88	2.64	2.64	(1.59-3.06)
AUMC	μgh^2ml^{-1}	11.25	24.61	16.5	24.75	17.48	17.4	(11.25-24.75)
MRT	h	4.58	8.03	10.38	8.60	6.618	8.03	(4.58-10.38)
MR	ratio	0.217	0.177	0.099	0.207	0.114	0.117	(0.099-0.217)
C_{max}	$\mu g.ml^{-1}$	0.421	0.315	0.231	0.309	0.46	0.315	(0.231-0.460)
t_{max}	h	1.5	3.0	6.0	6.0	3.0	3.0	(1.5-6.0)

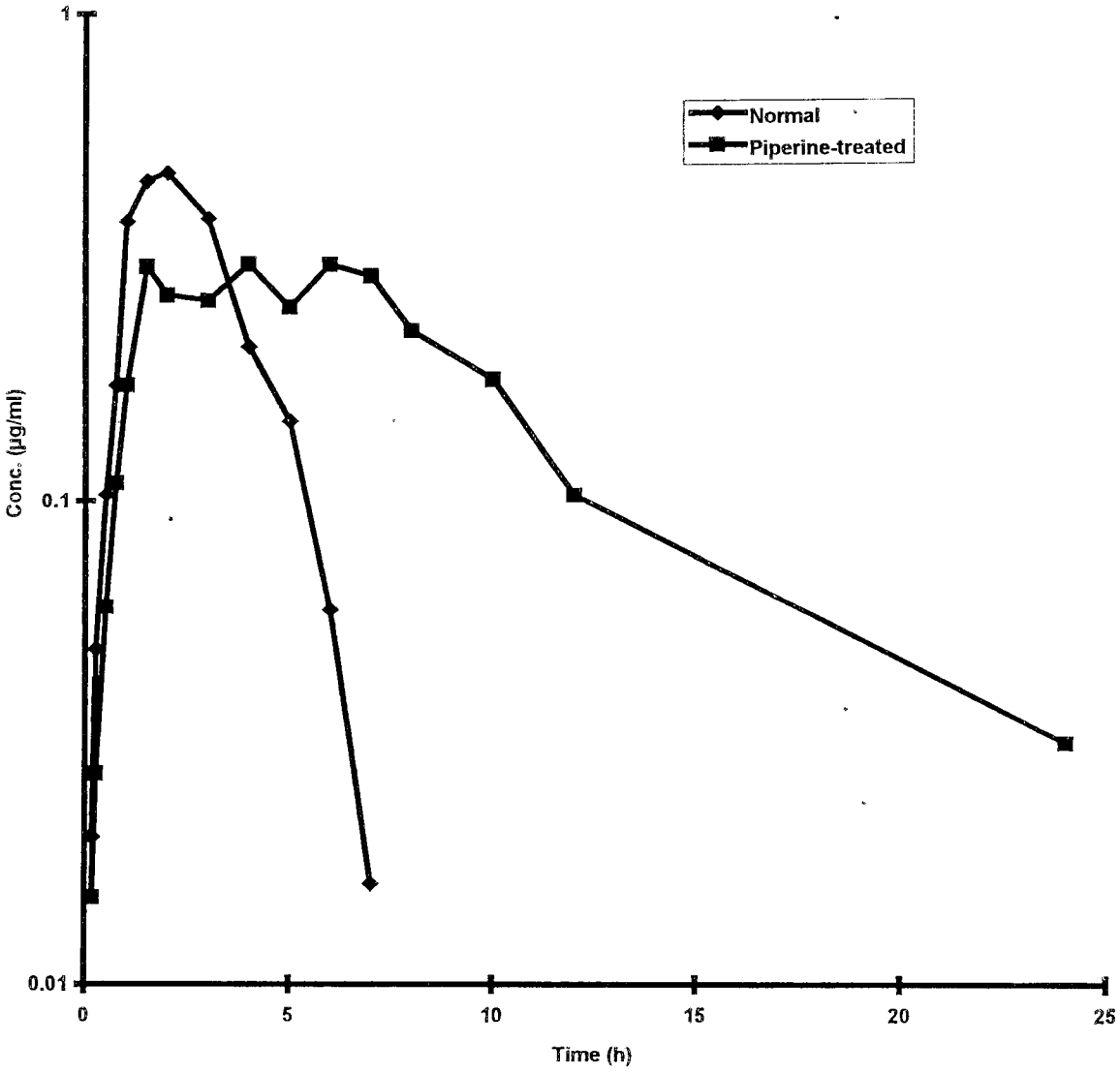

Fig. 16 Semilogarithmic plot of ciprofloxacin concentration in plasma vs. time following subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal and piperine-treated goats

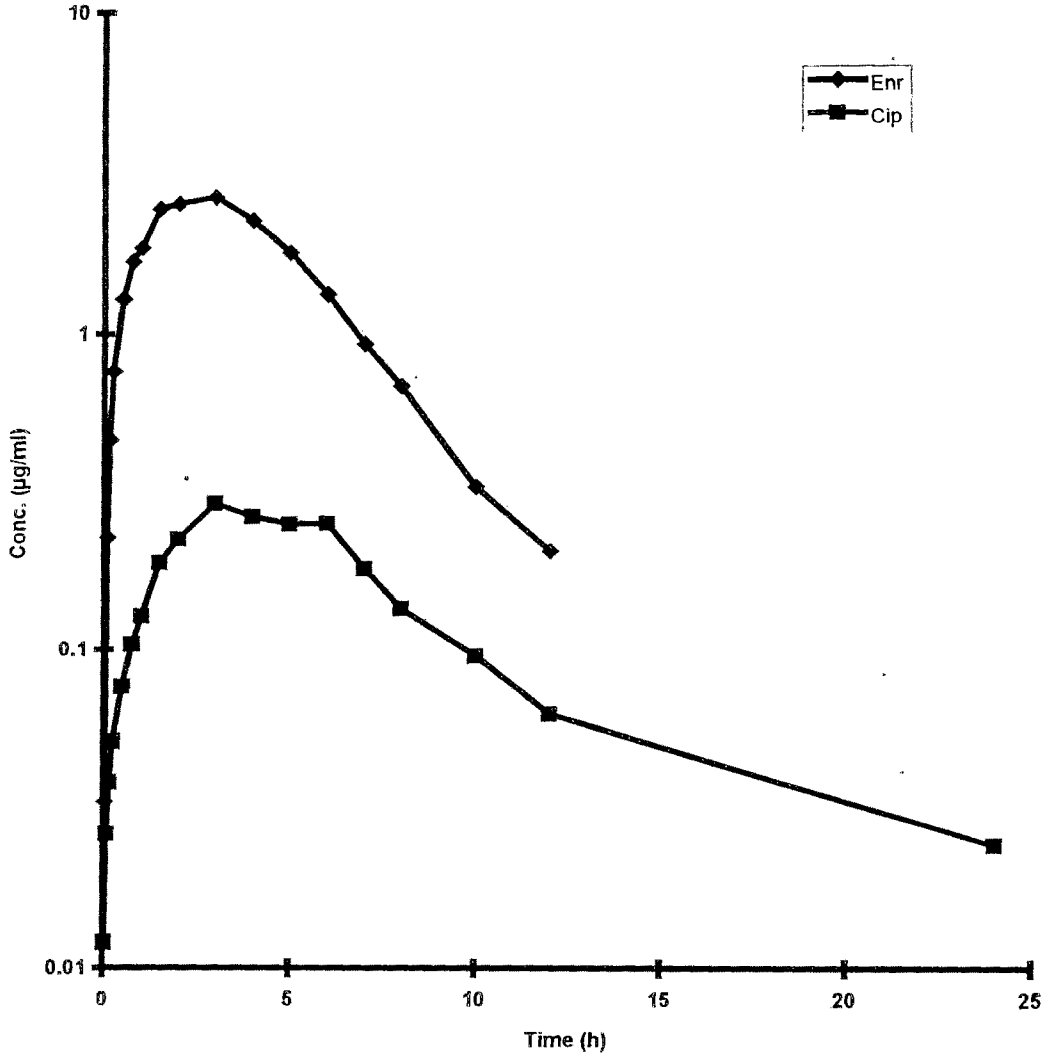

Fig. 17 Semilogarithmic plot of enrofloxacin and ciprofloxacin concentrations in plasma vs. time following concurrent administration of enrofloxacin (5 $mg.kg^{-1}$, s.c.) and probenecid (40 $mg.kg^{-1}$, s.c.) in goats

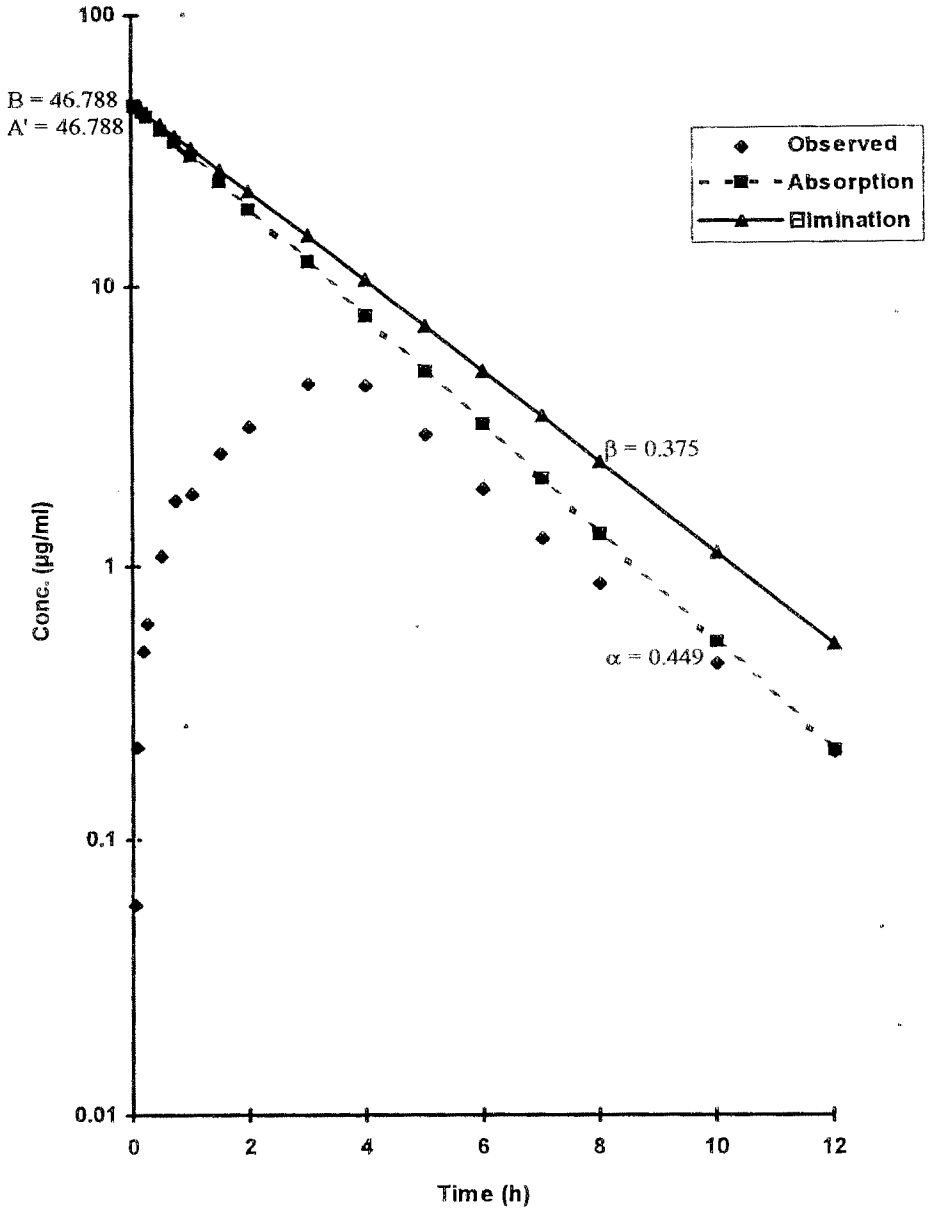

Fig. 18 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following concurrent administration of enrofloxacin (5 mg.kg^{-1},s.c.) and probenecid (40 mg.kg^{-1}. s.c.) in goat no.5

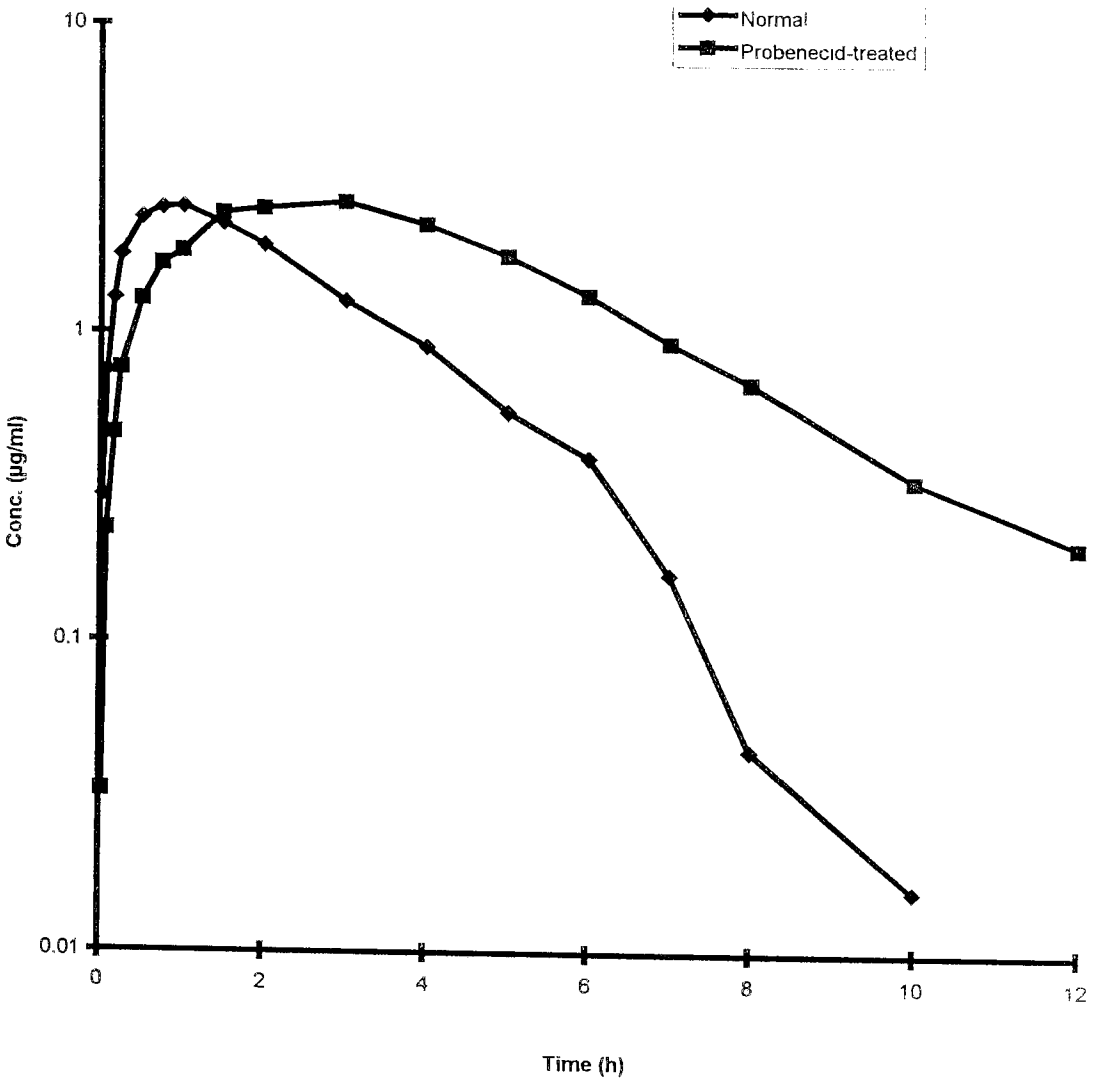

Fig. 19 Semilogarithmic plot of enrofloxacin concentration in plasma vs. time following subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal and probenecid-treated goats

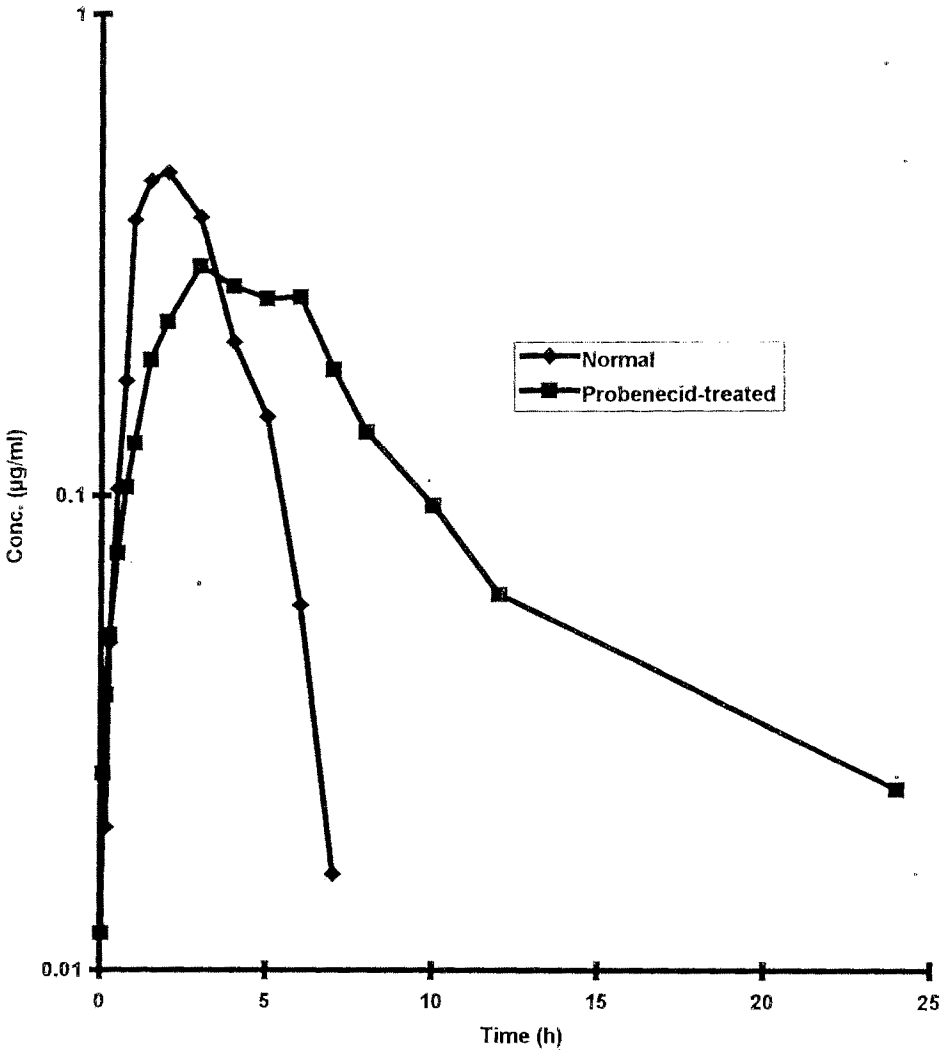

Fig. 20 Semilogarithmic plot of ciprofloxacin concentration in plasma vs. time following subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$) in normal and probenecid-treated goats

The important pharmacokinetic parameters of ENR in probenecid-treated goats were as follows : $t_{1/2\,ka}$, 1.28 (0.47-1.876) h, $t_{1/2\,\beta}$, 1.848 (1.269-2.911) h, AUC, 15-91 (11.34-23.14) $\mu g.h.ml^{-1}$, MRT, 4.44 (2.526-6.528) h, Cl_B, 314.16 (216.06 to 440.8) $ml.h^{-1}.kg^{-1}$ and F, 159.9 (130.6 - 316.7) %. The pharmacokinetic parameters of CIP were : $t_{1/2\,kf}$, 1.764 h, $t_{1/2\,\beta}$, 3.314 h, AUC, 2.64 $\mu g.h.ml^{-1}$ and MR, - 0.177.

As compared to normal goats, significant differences were observed in the values of AUC, AUMC, Cl_B and F in probenecid-treated animals, while for CIP, the parameters such as $t_{1/2\,kf}$, $t_{1/2\,\beta}$, AUC, MRT and MR differed significantly (Table 32).

4.6 Plasma concentrations and pharmacokinetics of ciprofloxacin after its single intravenous administration (7.5 $mg.kg^{-1}$) in goats

The plasma concentrations of CIP at various time intervals in animals of phase VI of the study are presented in Table 33. After i.v. administration, peak level of CIP was observed at 2 min (13.162 ± 1.453 $\mu g.ml^{-1}$) which rapidly declined to 4.191 ± 0.267 $\mu g.ml^{-1}$ at 0.5 h and then declined gradually to 0.115 ± 0.02 $\mu g.ml^{-1}$ at 8 h. In one animal, the CIP concentration was detectable upto 12 h.

The mean plasma concentrations of CIP are graphically depicted in Fig. 21. The pharmacokinetics of CIP was calculated by using a non-compartmental approach, and the pharmacokinetic parameters are presented in Table 34. The median elimination half-life was 1.435 (1.312-1.550) h. The AUC, MRT, $V_{d\,area}$ and Cl_B were : 11.29 $\mu g.h.ml^{-1}$, 1.843 h, 1.258 $L.kg^{-1}$ and 664.2 $ml.h^{-1}.kg^{-1}$, respectively. The median $V_{d\,SS}$ was 1.224 $L.kg^{-1}$.

Table 32. Effect of probenecid on the pharmacokinetics of enrofloxacin and ciprofloxacin after concurrent administration of enrofloxacin (5 mg.kg^{-1},s.c.) and probenecid (40 mg.kg^{-1}, s.c.) in goats

Parameter	Unit	ENROFLOXACIN				CIPROFLOXACIN			
		Normal		Probenecid -treated		Normal		Probenecid -treated	
A'	μg ml^{-1}	**6.474**	(4.445-12.029)	**10.758**	(8.32-46.788)	–	–	–	–
B	μg ml^{-1}	**6.474**	(4.444-12.330)	**10.757**	(8.319-46.788)	–	–	–	–
K_a	h^{-1}	**2.194**	(0.717-3.081)	**0.54**	(0.369-1.474)	–	–	–	–
K_f	h^{-1}	–	–	–	–	**0.837**	(0.547-1.136)	**0.393***	(0.221-0.497)
β	h^{-1}	**0.533**	(0.384-0.696)	**0.375**	(0.238-0.550)	**0.550**	(0.423-0.632)	**0.209***	(0.129-0.348)
$t_{1/2\,ka}$	h	**0.316**	(0.225-0.966)	**1.284**	(0.47-1.876)	–	–	–	–
$t_{1/2\,kf}$	h					**0.827**	(0.61-1.266)	**1.764***	(1.395-3.148)
$t_{1/2\,\beta}$	h	**1.301**	(0.996-1.805)	**1.848**	(1.269-2.911)	**1.259**	(1.096-1.638)	**3.314***	(1.993-5.376)
AUC	μg.h.ml^{-1}	**6.58**	(5.73-12.69)	**15.91***	(11.34-23.14)	**1.77**	(1.10-1.95)	**2.64***	(1.59-3.06)
AUMC	μg.h^2.ml^{-1}	**19.28**	(12.37-35.85)	**91.23***	(28.66-103.89)	**4.09**	(3.39-7.05)	**17.4***	(11.25-24.75)
MRT	h	**2.43**	(1.984-3.021)	**4.440**	(2.526-6.528)	**3.07**	(2.315-3.642)	**8.03***	(4.58-10.38)
$V_{d\,area}$	L. kg^{-1}	**1.24**	(0.674-1.52)	**1.19**	(1.093-3.370)	–	–	–	–
Cl_B	ml. h^{-1} kg^{-1}	**759.87**	(394-872.6)	**314.16***	(216.06-440.8)	–	–	–	–
F	%	**104.3**	(61.73-127.5)	**159.9***	(130.6-316.7)	–	–	–	–
MR	ratio					**0.192**	(0.110-0.296)	**0.177***	(0.099-0.217)
C_{max}	μg.ml^{-1}	**2.819**	(1.403-3.88)	**2.534**	(2.077-4.426)	**0.709**	(0.342-0.978)	**0.315***	(0.231-0.460)
t_{max}	h	**1.0**	(0.75-1.5)	**3.0**	(1.5-3.0)	**1.5**	(1.0-3.0)	**3.0**	(1.5-6.0)
MAT	h	**1.237**	(0.009-1.466)	**3.247***	(0.911-5.169)	–	–	–	–

Values are expressed as **Median** (range) of five animals

*Significantly different ($P<0.05$) from respective normal values

Table 33. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after single intravenous administration of ciprofloxacin (7.5 $mg.kg^{-1}$) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	8.826	15.102	13.785	17.022	11.075	13.162 ± 1.453
0.083	6.800	6.025	5.756	6.980	7.340	6.580 ± 0.297
0.167	6.151	5.827	3.797	5.158	5.905	5.368 ± 0.425
0.25	5.394	4.675	3.686	4.909	5.797	4.892 ± 0.359
0.5	4.147	4.281	3.312	4.225	4.991	4.191 ± 0.267
0.75	3.469	3.857	3.055	3.061	4.182	3.525 ± 0.221
1.0	3.011	3.411	2.608	2.698	3.397	3.025 ± 0.169
1.5	2.487	2.551	2.242	1.983	2.832	2.419 ± 0.144
2	1.728	2.136	1.467	1.469	2.800	1.920 ± 0.252
3	1.093	1.436	0.920	0.970	1.527	1.189 ± 0.123
4	0.807	0.881	0.722	0.719	0.783	0.782 ± 0.030
5	0.529	0.604	0.385	0.461	0.542	0.504 ± 0.037
6	0.335	0.412	0.251	0.362	0.323	0.337 ± 0.026
7	0.188	0.244	0.149	0.224	0.135	0.188 ± 0.02
8	0.118	0.158	0.070	0.161	0.068	0.115 ± 0.02
10	0.056	0.097	-	0.017	-	0.034 ± 0.01
12	-	0.038	-	-	-	0.007 ± 0.007

Table 34. Pharmacokinetics of ciprofloxacin after single intravenous administration of ciprofloxacin (7.5 mg.kg^{-1}) in goats

		Goat Number						
Parameter	Unit	1	2	3	4	5	Median	(range)
β	h^{-1}	0.459	0.447	0 483	0.528	0.512	0.483	(0.447-0.528)
$t_{1/2\beta}$	h	1.509	1.550	1.435	1.312	1.353	1.435	(1.312-1.550)
AUC	$\mu g.h.ml^{-1}$	11.27	13.52	9.63	11.29	13.26	11.29	(9.63-13.52)
AUMC	μgh^2ml^{-1}	23.06	28.52	17.35	20.46	24.27	23.06	(17.35-28.52)
MRT	h	2.046	2.11	1.803	1.843	1.83	1.843	(1.803-2.11)
$V_{d\,area}$	L. kg^{-1}	1.45	1.241	1.612	1.258	1.109	1.258	(1.109-1.612)
Cl_B	ml. h^{-1} kg^{-1}	665.55	554.7	778.5	664.2	567.8	664.2	(554.7-778.5)
V_{dss}	L. kg^{-1}	1.361	1.17	1.403	1.224	1.039	1.224	(1.039-1.403)

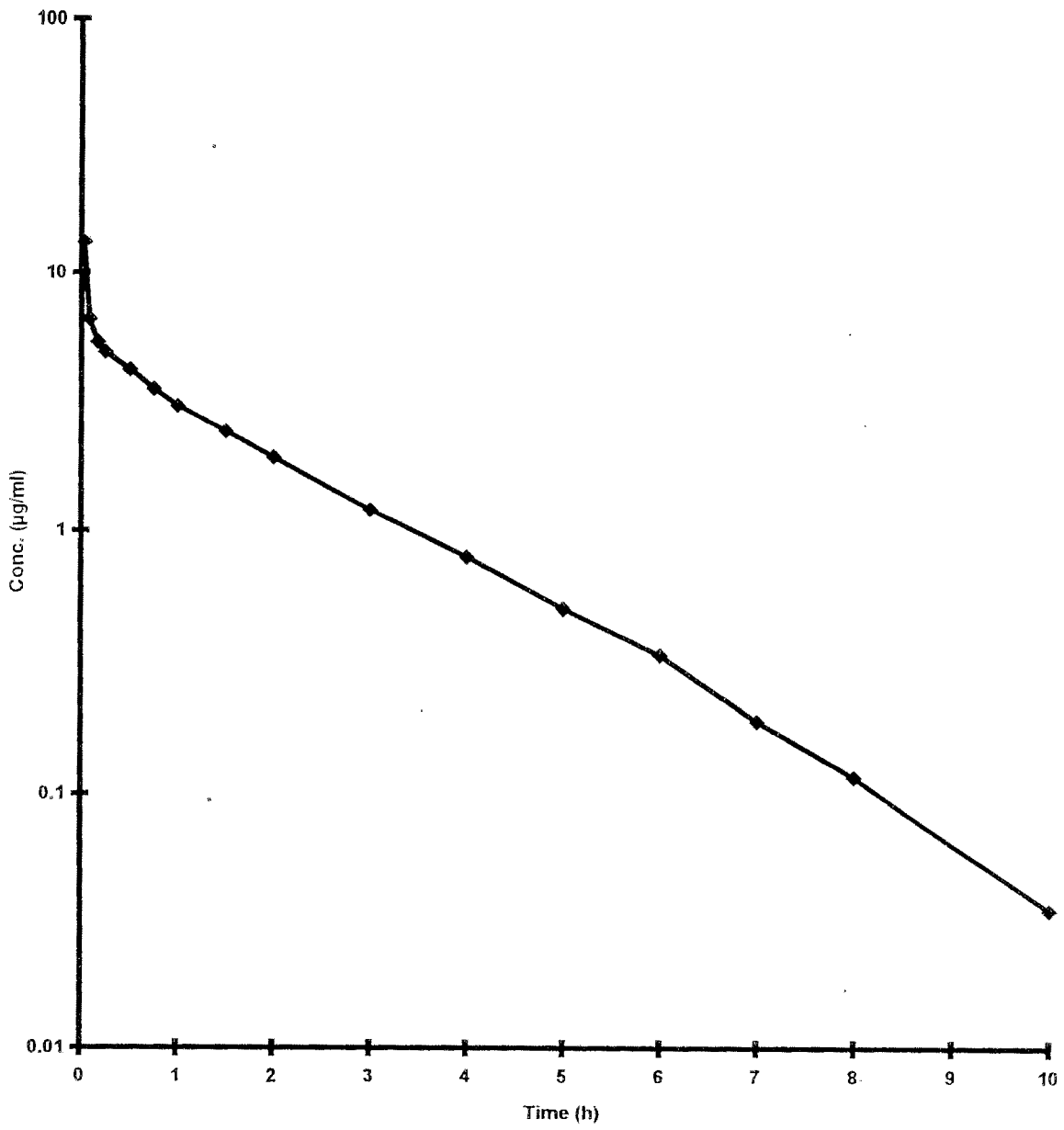

Fig. 21 Semilogarithmic plot of ciprofloxacin concentration in plasma vs. time following single intravenous administration of ciprofloxacin (7.5 $mg.kg^{-1}$)in goats

4.7 Plasma concentrations and pharmacokinetics of ciprofloxacin after its single subcutaneous administration (7.5 mg.kg^{-1}) in goats

The plasma concentrations of ciprofloxacin in animals of phase VII of the study are presented in Table 35 and the mean plasma concentration vs time data are graphically represented in Fig. 22.

After subcutaneous administration, CIP was detected in the plasma from 2 min. The median C_{max} was 1.787 μg.ml^{-1} observed at 10 min (t_{max}). From ten minutes, the concentrations started declining and CIP could be detected upto 12 h.

The pharmacokinetic variables of CIP were calculated by non-compartmental analysis and presented in Table 36. The median elimination half-life was 2.761 h. The median values of AUC, MRT, $V_{d\,area}$, Cl_B and F were 3.657 μg.h.ml^{-1}, 3.551 h, 2.694 L.kg^{-1}; 2050.4 ml.h^{-1}.kg^{-1} and 32.8%.

4.8 Plasma concentrations and pharmacokinetics of ciprofloxacin after co-administration of probenecid (40 mg.kg^{-1}, s.c.) and ciprofloxacin (7.5 mg.kg^{-1}, s.c.)

The plasma concentrations of ciprofloxacin in animals in phase VIII of the study are presented in Table 37 and the mean plasma concentration vs time data are graphically represented in Fig. 22.

After concurrent administration of ciprofloxacin and probenecid, CIP concentrations could be detected from 2 minutes. The peak plasma concentration (C_{max}) was 1.943 μg.ml^{-1} observed at 0.167 h (10 min). From 10 minutes, the concentrations started declining and CIP could be detected upto 24 h in all the animals.

Table 35. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after single subcutaneous administration of ciprofloxacin (7.5 $mg.kg^{-1}$) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	0.779	0.551	0.620	0.334	0.518	0.560 ± 0.072
0.083	1.663	1.117	1.440	1.185	1.289	1.339 ± 0.097
0.167	1.835	1.452	1.787	1.671	1.336	1.616 ± 0.096
0.25	1.753	1.222	1.589	1.792	1.251	1.521 ± 0.121
0.5	1.170	0.924	1.035	1.360	0.833	1.064 ± 0.093
0.75	1.121	0.783	0.872	0.937	0.770	0.897 ± 0.064
1.0	0.868	0.764	0.776	0.880	0.667	0.791 ± 0.039
1.5	0.707	0.574	0.442	0.612	0.511	0.569 ± 0.044
2	0.559	0.464	0.336	0.565	0.425	0.476 ± 0.038
3	0.325	0.355	0.244	0.426	0.310	0.332 ± 0.030
4	0.258	0.261	0.144	0.327	0.221	0.242 ± 0.03
5	0.249	0.238	0.135	0.217	0.175	0.203 ± 0.021
6	0.172	0.215	0.178	0.184	0.156	0.181 ± 0.009
7	0.140	0.160	0.156	0.177	0.159	0.158 ± 0.006
8	0.091	0.129	0.094	0.151	0.071	0.107 ± 0.014
10	0.057	0.118	0.071	0.104	0.054	0.081 ± 0.011
12	0.01	0.056	0.039	0.048	0.019	0.034 ± 0.009

Table 36. Pharmacokinetics of ciprofloxacin after single subcutaneous administration of ciprofloxacin (7.5 mg.kg^{-1}) in goats

Parameter	Unit	Goat Number 1	2	3	4	5	Median	(range)
β	h^{-1}	0.297	0.195	0.251	0.251	0.212	0.251	(0.195-0.297)
$t_{1/2\beta}$	h	2.33	3.554	2.761	2.761	3.268	2.761	(2.33-3.554)
AUC	$\mu g.h.ml^{-1}$	3.694	3.657	3.155	4.181	3.01	3.657	(3.01-4.181)
AUMC	μgh^2ml^{-1}	10.447	16.738	11.203	15.607	9.988	11.203	(9.98-16.738)
MRT	h	2.828	4.577	3.551	3.733	3.318	3.551	(2.828-4.577)
$V_{d\,area}$	$L.\ kg^{-1}$	2.242	2.839	3.106	2.694	2.667	2.694	(2.242-3.106)
Cl_B	$ml.\ h^{-1}\ kg^{-1}$	667.6	553.6	779.6	676.19	565.48	667.6	(553.6-779.6)
V_{dss}	$L.\ kg^{-1}$	5.742	9.386	8.441	6.696	8.208	8.208	(5.742-9.386)
F	%	32.8	27.0	32.8	37.0	22.7	32.8	(27.0-37.0)
C_{max}	$\mu g.ml^{-1}$	1.792	1.452	1.787	1.835	1.336	1.787	(1.336-1.835)
t_{max}	h	0.25	0.167	0.167	0.167	0.167	0.167	(0.167-0.25)
MAT	h	0.782	2.467	1.748	1.890	1.488	1.748	(0.782-2.467)
Cl_B/F	$ml.h^{-1}\ kg^{-1}$	2035.3	2050.4	2376.8	1793.6	2491.1	2050.4	(1793.6-2491.1)

Table 37. Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin after concurrent administration of ciprofloxacin (7.5 $mg.kg^{-1}$,s.c.) and probenecid (40 $mg.kg^{-1}$, s.c.) in goats

Time (h)	1	2	3	4	5	Mean ± SE
0.033	0.542	0.539	0.604	0.273	0.239	0.439 ± 0.076
0.083	1.540	0.917	1.816	0.892	0.828	1.198 ± 0.201
0.167	2.727	1.415	2.281	1.943	1.345	1.942 ± 0.261
0.25	2.652	1.050	1.720	1.692	1.251	1.673 ± 0.276
0.5	2.301	0.736	1.699	1.493	0.918	1.429 ± 0.281
0.75	1.800	0.727	1.647	1.346	0.878	1.280 ± 0.210
1.0	1.001	0.688	1.681	1.182	0.834	1.197 ± 0.198
1.5	1.363	0.546	1.273	0.952	0.701	0.948 ± 0.149
2	1.180	0.433	1.176	0.705	0.632	0.825 ± 0.151
3	1.022	0.368	0.844	0.516	0.494	0.649 ± 0.122
4	0.735	0.262	0.670	0.382	0.382	0.486 ± 0.091
5	0.589	0.214	0.561	0.395	0.326	0.417 ± 0.071
6	0.388	0.147	0.449	0.350	0.275	0.322 ± 0.052
7	0.343	0.125	0.325	0.324	0.191	0.262 ± 0.044
8	0.312	0.104	0.241	0.194	0.140	0.198 ± 0.037
10	0.182	0.087	0.152	0.162	0.101	0.137 ± 0.018
12	0.062	0.053	0.116	0.094	0.065	0.078 ± 0.011
24	0.017	-	0.042	0.055	0.017	0.026 ± 0.009

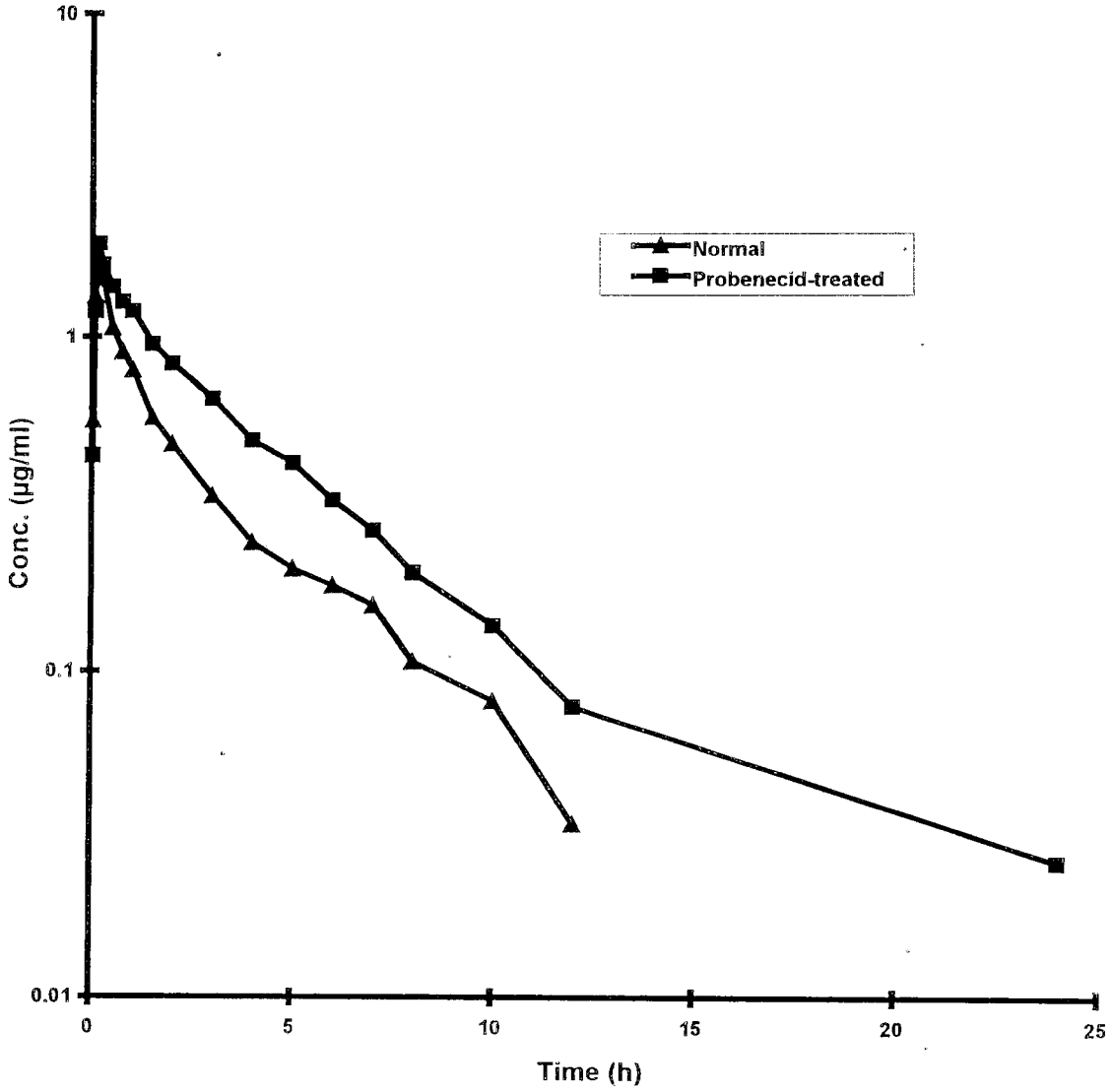

Fig. 22 Semilogarithmic plot of ciprofloxacin concentration in plasma vs. time following subcutaneous administration of ciprofloxacin (7.5 $mg.kg^{-1}$) in normal and probenecid-treated goats

The pharmacokinetic parameters of CIP obtained in probenecid-treated goats are presented in Table 39. The median elimination half-life was 3.5 h. The median values of AUC, MRT, $V_{d\,arca}$, Cl_B, F and MAT were 6.661 $\mu g.h.ml^{-1}$ 4.893 h, 3.798 $L.kg^{-1}$, 1125.9 $ml.h^{-1}.kg^{-1}$, 59.0% and 3.09 h, respectively.

The plasma levels and pharmacokinetics variables of CIP in probenecid-treated animals were compared with normal goats and results are summarized in Table 38 and 40.

As compared to normal animals, the plasma levels were appreciably higher in probenecid-treated goats from 0.5 h upto 10 h and significantly higher at 12 and 24 h. The pharmacokinetic parameters did not differ significantly (Table 40). However, probenecid administration produced appreciable increase in the values of $t_{1/2\,\beta}$, AUC, MRT, MAT and $V_{d\,arca}$ and decrease in Cl_B.

Table 38. Effect of probenecid (40 mg. kg^{-1}, s.c.) on the plasma concentrations of ciprofloxacin after single subcutaneous administration of ciprofloxacin (7.5 mg.kg^{-1}) in five goats

Time after drug administration (h)	Ciprofloxacin	
	Normal	probenecid-treated
0.033	0.560±0.072	0.439±0.076
0.083	1.339±0.097	1.198±0.201
0.167	1.616±0.096	1.942±0.261
0.25	1.521±0.121	1.673±0.276
0.5	1.064±0.093	1.429±0.281
0.75	0.897±0.064	1.280±0.210
1.0	0.791±0.039	1.197±0.108
1.5	0.569±0.044	0.948±0.149
2.0	0.476±0.038	0.825±0.151
3.0	0.332±0.030	0.649±0.122
4.0	0.242±0.030	0.486±0.091
5.0	0.203±0.021	0.417±0.071
6.0	0.181±0.009	0.322±0.052
7.0	0.158±0.006	0.262±0.044
8.0	0.107±0.014	0.198±0.037
10	0.081±0.011	0.137±0.018
12	0.034±0.009	0.078±0.011**
24	-	0.026±0.009

Values are expressed as µg of enrofloxacin/ciprofloxacin/ml of plasma and represent the Mean ± SE

** Significantly different ($P<0.01$) from respective normal values

Table 39. Pharmacokinetics of ciprofloxacin after concurrent administration of ciprofloxacin (7.5 mg.kg^{-1}, s.c.) and probenecid (40 mg.kg^{-1}, s.c.) in goats

		Goat Number						
Parameter	Unit	1	2	3	4	5	Median	(range)
β	h^{-1}	0.253	0.198	0.205	0.166	0.120	0.198	(0.120-0.253)
$t_{1/2\beta}$	h	2.739	3.5	3.380	4.174	5.775	3.5	(2.739-5.775)
AUC	$\mu g.h.ml^{-1}$	8.497	3.377	8.234	6.661	4.761	6.661	(3.377-8.497)
AUMC	μgh^2ml^{-1}	34.778	14.784	40.286	44.340	25.960	34.778	(14.784-44.34)
MRT	h	4.093	4.378	4.893	6.657	5.452	4.893	(4.093-6.657)
$V_{d\ area}$	$L.\ kg^{-1}$	2.630	2.804	3.798	4.002	4.699	3.798	(2.63-4.699)
Cl_B	$ml.\ h^{-1}\ kg^{-1}$	666.7	535.22	778.28	664.3	563.95	664.3	(535.2-778.3)
V_{dss}	$L.\ kg^{-1}$	3.612	9.722	4.456	7.495	8.589	7.495	(3.612-9.722)
F	%	75.4	25.0	85.0	59.0	35.8	59.0	(25.0-85.0)
C_{max}	$\mu g.ml^{-1}$	2.727	1.415	2.281	1.943	1.345	1.943	(1.415-2.727)
t_{max}	h	0.167	0.167	0.167	0.167	0.167	0.167	(0.167-0.167)
MAT	h	2.047	2.268	3.090	4.814	4.343	3.09	(2.047-4.184)
Cl_B/F	$ml.h^{-1}.kg^{-1}$	884.4	2220.8	710.26	1125.9	1575.28	1125.9	(884.4-2220.8)

Table 40. Effect of probenecid on the pharmacokinetics of ciprofloxacin after concurrent administration of ciprofloxacin (7.5 mg.kg^{-1}, s.c.) and probenecid (40 mg.kg^{-1}, s.c.) in goats

		CIPROFLOXACIN			
Parameter	Unit	Normal		Probenecid-treated	
β	h^{-1}	**0.251**	(0.195-0.297)	**0.198**	(0.120-0.253)
$t_{1/2\beta}$	h	**2.761**	(2.33-3.554)	**3.5**	(2.739-5.775)
AUC	$\mu g.h.ml^{-1}$	**3.657**	(3.01-4.181)	**6.661**	(3.377-8.497)
AUMC	$\mu g.h^2.ml^{-1}$	**11.203**	(9.988-16.738)	**34.778**	(14.784-44.340)
MRT	h	**3.551**	(2.828-4.577)	**4.893**	(4.093-6.657)
$V_{d\,area}$	L. kg^{-1}	**2.694**	(2.242-3.106)	**3.798**	(2.630-4.699)
Cl_B	ml. h^{-1} kg^{-1}	**667.6**	(553.6-779.6)	**664.3**	(535.2-778.3)
F	%	**32.8**	(27.0-39.0)	**59.0**	(25.0-85.0)
Cl_B/F	ml. h^{-1} kg^{-1}	**2050.4**	(1793.6-2491.1)	**1125.9**	(884.4-2220.8)
C_{max}	$\mu g\ ml^{-1}$	**1.787**	(1.336-1.835)	**1.943**	(1.415-2.727)
t_{max}	h	**0.167**	(0.167-0.250)	**0.167**	(0.167-0.167)
MAT	h	**1.748**	(0.782-2.467)	**3.09**	(2.047-4.814)
Vd_{SS}	L. kg^{-1}	**8.208**	(5.742-9.386)	**7.495**	(3.612-9.722)

Values are expressed as **Median** (range) of five animals.

DISCUSSION

Fluoroquinolones constitute an important group of antibacterial agents that are increasingly being used in the combat against infections. Unlike their precursors such as nalidixic acid, fluoroquinolones have shown a multifold increase in their intrinsic bactericidal activity and tissue penetrability (Boothe, 1994). Enrofloxacin was the first fluoroquinolone approved exclusively for veterinary use in 1988. Enrofloxacin, is converted to ciprofloxacin by de-ethylation *in vivo*. Ciprofloxacin which is marketed for use in humans, is not approved for use in animals. However, it is commonly being used in veterinary medicine (Boothe, 1994).

The susceptibility pattern of organisms to enrofloxacin is parallel to that of ciprofloxacin. Both are bactericidal and penetrate well into tissues. Owing to their broad spectrum of activity, they are used in a variety of infections caused by gram positive and gram negative bacteria and mycoplasmas.

The success of antimicrobial therapy depends on the use of optimal dosage regimen of drug(s). The computation of an optimal dosage regimen depends on the clear understanding of the disposition of the drugs in the target species. While most of the pharmacokinetic studies are conducted in healthy animals, their actual utility is in clinically ill animals. Various cardinal signs of infection and inflammation such as fever itself can modify the kinetic behaviour of drugs by altering their absorption, distribution or elimination. In such conditions the dosage regimen may have to be modified and hence it is important to study the disposition kinetics of drugs in diseased animals. Since fever, is one of the cardinal signs of inflammation and infection, in this study a febrile condition was induced in goats by injecting endotoxin (LPS) and the pharmacokinetics of enrofloxacin was studied in febrile animals.

Bioavailability refers to the fraction of drug absorbed. Higher bioavailability indicates longer persistence of the drug in the body which would mean longer dosing intervals and fewer drug administrations. There has been a persistent attempt at increasing the bioavailability of drugs by modification of processes such as drug absorption, metabolism or elimination. In this study, two different agents known to enhance the bioavailability of coadministered drugs have been used.

Probenecid, an organic anion transport inhibitor, is known to block renal excretion of drugs. Although it is used in combination with penicillins and cephalosporins to increase the bioavailability of these drugs, little is known about its effects on flouroquinolones pharmacokinetics. In this study, the effect of probenecid on the kinetics of enrofloxacin and ciprofloxacin after subcutaneous administration was investigated.

Piperine, an alkaloid from pepper, is an active ingredient of trikatu. It is a common ingredient of many ayurvedic preparations and has been attributed with multiple activities. One of the activities of piperine that has been reported to be of

great interest is its ability to enhance the bioavailability of some drugs. Though there are many studies on piperine in humans and laboratory animals, to our knowledge, no report on piperine-antimicrobial drug interaction is available in any ruminant species. In this study, the effect of piperine on the bioavailability of enrofloxacin and its metabolite ciprofloxacin was investigated after concomitant subcutaneous administration of piperine and enrofloxacin.

The present investigation, thus comprised of (1) pharmacokinetic studies of enrofloxacin and its metabolite ciprofloxacin after intravenous or subcutaneous administration of enrofloxacin (5 $mg.kg^{-1}$); (2) studies on the effect of febrile state, concurrent administration of piperine or probenecid on enrofloxacin/ciprofloxacin pharmacokinetics after subcutaneous administration; (3) disposition kinetic studies of ciprofloxacin after its intravenous or subcutaneous administration (7.5 $mg.kg^{-1}$) and (4) studies on the effect of probenecid coadministration on ciprofloxacin pharmacokinetics in goats.

5.1. Plasma concentrations and pharmacokinetics of enrofloxacin and its metabolite, ciprofloxacin after single intravenous administration of enrofloxacin (5 mg kg^{-1}) in goats

In the present study, enrofloxacin was administered intravenously in normal goats at a dose rate of 5 mg kg^{-1}. The same dose of enrofloxacin has been used in other species such as lactating cows (Kaartinen *et al.*, 1995; Malbe *et al.*, 1996), rabbits (Broome *et al.*, 1991), horses (Giguere *et al.*, 1996; Kaartinen *et al.*, 1997a), llamas (Christensen *et al.*, 1996) and dogs (Kung *et al.*, 1993).

Following intravenous administration, initial plasma concentration of 11.66 ± 1.48 µg. ml^{-1} was observed at 0.033 h and the minimum detectable level was 0.029 ± 0.029 at 8 h. Kaartinen *et al.* (1995) also reported detectable antimicrobial activity in lactating cows upto 8 h, whereas in rabbits detectable concentrations

were observed upto 10 h (Broome *et al.*, 1991). Peak plasma level of CIP observed in the present study was 1.159 μg ml^{-1} at 1.5 h after i.v. administration of enrofloxacin. The C_{max} obtained in the present study was higher than that obtained in llamas (0.42 μg ml^{-1}, Christensen *et al.*, 1996) and dairy cows (0.55 μg ml^{-1}, Malbe *et al.*, 1996).

Based on plasma levels of ENR and CIP, their respective pharmacokinetic parameters were calculated. After intravenous administration, the disposition of enrofloxacin conformed to a two- compartment open model. The high value of distribution rate constant (6.217 h^{-1}) and relatively low elimination rate constant (0.664 h^{-1}) obtained in this study, are suggestive of a rapid distribution phase, followed by a slower elimination phase. The distribution half life obtained in the present study was 0.134 h (0.011-262). Shorter distribution half life of enrofloxacin, (t_{112a} , 5.1 min) has been reported in dairy cows (Malbe *et al.*, 1996) . Conversely, longer distribution half-life of 0.67 h for enrofloxacin has been reported in rabbits (Broome *et al.*, 1991).

Ciprofloxacin is formed as a metabolite of enrofloxacin in almost all the species. However, marked inter-species variation has been observed in the extent and rate of ciprofloxacin formation. In the present study, the mean k_f value obtained was 1.04 h^{-1} and the $t_{1/2\,kf}$ was 0.667 h (0.29-0.963). However, a shorter metabolite formation half life ($t_{1/2\,kf}$, 13.2 min) has been reported in dairy cows (Malbe *et al.*, 1996).

Elimination half life ($t_{1/2\,\beta}$) of ENR obtained in goats, in the present study, was 1.157 h. Almost a similar $t_{1/2\beta}$ of 65 minutes was obtained in dairy cows (Malbe *et al.*, 1996). However, Kaartinen *et al.*, (1995) reported longer $t_{1/2\beta}$ of 1.7 h in lactating cows. The elimination half lives of ENR after intravenous administration of enrofloxacin were: 6.6 h and 4.9 h in calves (Kaartinen *et al.*, 1997b), 16.31 h in Holstein-Friesian calves (Martinez-Larranaga *et al.*, 1997), 3.73 h in pigs (Pijpers *et al.*, 1997), and 3.73 and 3.8 h in sheep (Mengozzi *et al.*, 1996; Pozzin *et al.*,

1997). The elimination half life obtained in the present study indicates that goats tend to eliminate ENR faster than other animal species.

The elimination half life of ciprofloxacin as a metabolite of ENR, was 0.815 h (0.392-1.330 h). Longer elimination half lives have been reported in other species such as 4.8 h in sheep (Mengozzi *et al.*, 1996) 4.4 h in horses (Kaartinen *et al.*, 1997a), 3.9 h and 4.79 h in dogs (Kung *et al.*, 1993 and Cester *et al.*, 1996), and 160 min in dairy cows (Malbe *et al.*, 1996). Based on the results obtained in this study, it can be concluded that like ENR, CIP is also eliminated rapidly in goats than in other animal species.

The area under the concentration-time curve (AUC) is a very useful parameter in pharmacokinetics. It forms the basis for calculation of other non compartmental kinetic parameters such as MRT, Cl_{β}, $V_{d\,area}$ etc. It is also employed for computation of bioavailability of drugs administered by various extravascular routes.

The AUC value obtained in the present study, for ENR was 9.95 (5.45-11.31 $\mu g.h.ml^{-1}$) after its single intravenous administration (5 mg kg^{-1}). The AUC values ($\mu g.h.ml^{-1}$) obtained in llamas (6.95 ± 0.93; Christensen *et al.*, 1996) and lactating cows (7.42 ± 0.02; Malbe *et al.*, 1996) were lower than that obtained in the present study.

The AUC value of CIP obtained in the present study was 2.38 (1.68-5.47μg h. ml^{-1}). The metabolite ratio (MR) of ciprofloxacin, which was calculated as a ratio of AUC of the metabolite to the AUC of the parent compound was 0.308 or 30.8%. MR of CIP has been calculated similarly and reported in other species. In sheep, MR was 36% (Mengozzi *et al.*, 1996). Relative ly lower value (29%) was observed in dairy cows (Malbe *et al.*, 1996). Higher percentage of 43% has been reported in dogs (Cester *et al.*, 1996) and 36% in llamas (Christensen *et al.*, 1996) Kaartinen *et al.* (1997a) reported MR of CIP as 20-35% in horses. However, a very low conversion of <10% has been reported in ducks (Intorre *et al.*, 1997).

Apparent volume of distribution is important to assess the extent of distribution of a drug in body fluids and tissues. The $V_{d\ area}$ obtained in the present study was 0.863 L-kg^{-1}. In calves, the $V_{d\ area}$ was reported to be 1.19 L.kg^{-1} (Garcia *et al.*, 1996). However larger $V_{d\ are}$ of 2.12 and 3.45 L.kg^{-1} have been reported in rabbits and pigs, respectively (Broome *et al.*, 1991; Zeng *et al.*, 1996). Fluoroquinolones, in general, have excellent tissue penetration as reflected by high $V_{d\ aren}$ in the present study, too.

$V_{d\ ss}$ provides an estimate of drug distribution that is independent of elimination processes. It is a function of a drug's affinity for peripheral tissues. In the present study, the $V_{d\ ss}$ obtained for enrofloxacin after intravenous administration at a dose rate of 5 mg.kg^{-1} was 0.850 L.kg^{-1} (0.528-1.064). The $V_{d\ ss}$ of ENR in rabbits was reported to be 0.93 L.kg^{-1} (Broome *et al.*, 1991) which is comparable to the results obtained in this study. However, higher $V_{d\ ss}$ values have been documented in sheep (3.02 L.kg^{-1}, Mengozzi *et al.* , 1996) and pigs (3.13 L.kg^{-1}, Pijpers *et al.*.1997).

Clearance (Cl_B) is another important pharmacokinetic parameter that is a characteristic of a drug. The Cl_B obtained in the present study was 502.53 ml h^{-1} kg^{-1}. A comparable value of 606. ml h^{-1} kg^{-1} has been documented in rabbits (Broome *et al.*, 1991). Low clearance for ENR has been reported in pigs (0.37 L. h^{-1}kg^{-1}; Nielsen and Hansen, 1997) and calves (3.8 ml. min $^{-1}$ kg^{-1}; Garcia *et al.*, 1996). However, much higher clearance of 21 ml.min^{-1} kg^{-1} has been observed in dairy cows (Malbe *et al.*, 1996).

Mean residence time is the mean time required for a drug molecule to traverse through the body and thus reflects time associated with absorption, distribution and elimination. In the present study, MRT value obtained for ENR was 1.359 h (0.927-1.974). The MRT value obtained for the metabolite CIP was 2.02 h (0.95-2.630 h). Longer MRT of ENR and CIP have been obtained in sheep (ENR,

5.36 ± 0.764 h; CIP 8.35 ± 1.68 h) after intravenous administration of ENR (Mengozzi *et al.*, 1996). In lactating cows, Kaartinen *et al.*(1995) reported a MRT of 1.80 h, which is comparable to that obtained in the present study in goats. Nielsen and Hansen (1997) reported a very high MRT of 11.0 ± 3.0 h in pigs, whereas, in llamas MRT reported was 4.95 ± 2.87 h (Christensen *et al.*, 1996). From these data, it appears that the persistence of ENR and CIP is much shorter in goats as compared to other species.

5.2 Plasma concentrations and pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after single subcutaneous administration of enrofloxacin (5 mg kg^{-1})

In phase II of the present study, enrofloxacin was administered subcutaneously at the dose of 5 mg kg^{-1}. The same dose has been used for determining the pharmacokinetics of enrofloxacin after intramuscular administration in horses (Kaartinen *et al.*, 1997a), rabbits (Cabanes *et al.*, 1992), buffalo bulls (Verma *et al.*, 1999) and goats (Rao, 1999). Pharmacokinetics of enrofloxacin has also been investigated after its subcutaneous administration at a dose of 5 mg kg^{-1} body weight in rabbits and lactating cows (Broome *et al.*, 1991;Kaartinen *et al.*, 1995).

Detectable concentrations of enrofloxacin (0.295 ± 0.194 µg ml^{-1}) were found at 2 minutes post-administration. The peak plasma concentration of enrofloxacin, after its single subcutaneous administration (5 mg kg^{-1}) was 2.819 µg ml^{-1} (1.403-3.880) which occurred at 1.0 h.

Enrofloxacin concentration could be detected in plasma upto 6 h in one animal, upto 7 h in two animals, upto 8 h in one animal and in the last animal upto 10 h. The peak plasma level obtained in this study was similar to the Cmax (2.8 ± 0.289 µg ml^{-1}) reported after intramuscular administration of ENR at the same dose rate in goats (Rao, 1999). After s.c. administration of ENR in rabbits, Broome *et al.*

(1991) reported a slighty lower C_{max} (2.07 μg ml^{-1}). The time to maximum plasma concentration (t_{max}) obtained in the present study was 1.0 h , with a range of 0.75-1.5 h. This was comparable to the t_{max} obtained in goats after i.m. administration of ENR (0.875 ± 0.55 h) and the t_{max} in rabbits after s.c. administration (0.88 h; Broome *et al.*, 1991).

After s.c. administration of ENR, the metabolite ciprofloxacin was detected at 0.167 h in two animals and from 0.5 h in all other animals. The C_{max} of CIP was 0.709 μg ml^{-1} (0.342-0.978 μg.ml^{-1}) observed at 1.5 h . The concentrations of CIP were detected upto 6-7 h. After i.m. administration of ENR (5 mg kg^{-1}), Rao (1999) reported a much lower C_{max} of CIP (0.238 ± 0.017 μg ml^{-1}) at 1.2 ± 0.22 h (t_{max}).

After single subcutaneous administration of enrofloxacin, the disposition pattern obtained was described by a one compartment open model. The absorption rate constant (K_a) was 2.194 h^{-1} and the absorption half life was 0.316 h, which is quite similar to the value obtained after intramuscular administration (0.283 ± 0.024 h) in goats (Rao, 1999). The metabolite formation half life for CIP (0.827 h) was also comparable to that obtained after i.m. administration of ENR in goats (Rao, 1999). The results suggest that similar absorption and metabolite formation pattern after both subcutaneous and intramuscular administration of ENR in goats.

The elimination half life ($t_{1/2\beta}$) of ENR obtained in the present study (1.353 h) after subcutaneous administration was markedly shorter than that reported in cross-bred calves (19.07 h; Martinez - Larranaga *et al.*, 1997) and slighty shorter than the $t_{1/2\beta}$ reported in rabbits (1.71 h ; Broome *et al.*, 1991). However, in goats, Rao (1999) reported a $t_{1/2\beta}$ of 1.396 h after i.m. administration which is almost similar to the value obtained in this study. The elimination half life of CIP obtained in this study (1.259 h) was shorter than the 1.819 h reported after i.m. administration of ENR in goats (Rao, 1999).

The area under the plasma concentration-time curve (AUC) of ENR after its s.c. administration was 6.58 $\mu g.h.ml^{-1}$ (5.73-0 12.69 $\mu g.h.ml^{-1}$) and that of the active metabolite CIP was 1.77 $\mu g.h.ml^{-1}$. The AUC of ENR obtained in this study is comparable to that reported for cattle (7.1 $\mu g.h.ml^{-1}$) by HPLC assay procedure after s.c. administration of 7.5 $mg.kg^{-1}$ dose (Stegemann *et al.*, 1997). When determined by microbiological assay, the same workers reported a higher value of AUC of 18.9 $\mu g.h.ml^{-1}$. In rabbits, given ENR (5 $mg.kg^{-1}$,s.c.) Broome *et al.* (1991) reported an AUC of 6.09 $\mu g\ ml^{-1}$ which is comparable to the results obtained in the present study. An AUC of 7.516 $\mu g.h.ml^{-1}$ has been reported after i.m. administration of ENR (5 $mg.kg^{-1}$) in goats (Rao, 1999).

The bioavailability of ENR after s.c administration was compared by the ratio AUC s.c. /AUC i.v. In this study, a median bioavailability of 104.3% was obtained, suggesting complete absorption of the drug following s.c. administration. This value is in complete agreement with the reported bioavailability values in cattle (110%; Stegemann *et al.* , 1997) and calves (96%; Martinez-Larranaga *et al.* , 1997) after subcutaneous administration of enrofloxacin. The bioavailability was 119% after i.m. administration in goats (Rao 1999). The results suggest that s.c. route is appropriate for administration of ENR in goats.

The metabolite ratio (MR) of CIP in the present study, was 0.177 which is lower as compared to the MR (0.34) obtained after i.m. administration (Rao, 1999).

5.3 Plasma concentrations and pharmacokinetics of ENR and CIP after single subcutaneous administration of Enrofloxacin (5 mg. kg^{-1}) in febrile goats

The effect of endotoxin-induced fever was investigated on the plasma concentrations of ENR and CIP and their pharamacokinetics in goats.

In febrile animals, the plasma concentration of ENR did not differ significantly when compared to normal goats. However, there was higher

concentration of ENR and CIP in the later samples with the drug concentrations detectable up to 12 h as compared to 10 h in normal goats. The pharmacokinetic parameters determined in febrile goats did not differ significantly from that of normal animals.Nevertheless, appreciably higher values of AUC and MRT of ENR were obtained indicating higher persistence of the drug in febrile goats.. This may be attributed to the diminished metabolism of ENR in febrile goats. Endotoxin exposure is known to alter xenobiotic metabolism in the hepatocytes (Roth *et al.*, 1997). This is further confirmed by the fact, that in febrile goats sustained lower concentrations of CIP, a metabolite of ENR, were measured initially. There was also an appreciable decrease in C_{max} and a delayed t_{max} of CIP. Similar findings of reduced metabolism including hydroxylation of sulphadimidine and glucuronidation of chloramphenicol have been reported in febrile goats (Nouws *et al.*, 1986; Anika *et al.*, 1986).

The clearance (Cl_B) of the parent drug (ENR) was also appreciably decreased in febrile goats. This could be due to reduction in the blood flow to kidney and liver following endotoxin exposure, since clearance of a drug is dependent on the perfusion of these organs by blood. However, the C_{max} and t_{max} of ENR did not vary significantly. The bioavailability of ENR, too, did not vary significantly in febrile goats,

Despite a few alterations in the pharmacokinetics, the results suggest that alterations in the dosage regimen of ENR may not be required for febrile goats, when the drug is administered by subcutaneous route.

5.4 Effect of piperine on the plasma concentrations and pharmacokinetics of ENR and CIP in goats

Piperine is a natural alkaloid, isolated from pepper. Owing to many reports of its ability to increase the bioavailability of co-administered drugs, it was envisaged

to study the effect of piperine on the disposition kinetics of ENR. Studies so far carried out with piperine are limited to human and rats and as such no report is available in domestic animals. A suitable dosage of piperine was calculated for goats by extrapolation of rat dosage using the Km factor (van Miert, 1986). Km factor is arrived at based on body weight to surface area ratios in different species of animals. The Km factors attributed to rats and goats are 6 and 45.5, respectively. Thus dividing the dosage used in rats (10 mg kg^{-1}) by the ratio of Km factor of rats to goats (6/.45.5) yielded dose of 10 x 0.13 = 1.3 mg kg^{-1} . Accordingly, an appropriate dose of piperine (2 mg.kg^{-1}) was used in this study. Piperine, prepared as 1.25% solution was injected subcutaneously at a dose of 2 mg kg^{-1} . In piperine co-administered goats the plasma concentrations of ENR were significantly ($P<0.01$) higher from 5 h to 10 h . While, in normal goats, ENR could not be detected beyond 10 h, in piperine -treated goats, detectable concentration of ENR were found upto 24 h. This is suggestive of longer persistence of ENR in piperine-treated goats Important pharamacokinetic parameters of ENR such as $t_{1/2\beta}$, MRT and AUC and F were significantly increased indicating higher availability of ENR in piperine-treated goats. A significant increase in AUC of rifampicin (47.45 µg.h.ml^{-1} in piperine-untreated versus 81 µg.h.ml^{-1} in piperine-treated) has been reported on co-administration of piperine (50 mg total dose) in humans (Zutshi *et al.*, 1985). Piperine (10 mg kg^{-1}, p.o) has been shown to enhance plasma concentrations, $t_{1/2\beta}$ and AUC of phenytoin in human volunteers (Bano *et al.*, 1986). Recently, co-administration of piperine has been demonstrated to significantly increase the values of AUC, C_{max} , t_{max} and $t_{1/2\beta}$ of oxyphenbutazone in rats (Majumdar, *et al.*, 1999).

Atal *et al.*, (1985) studied the effects of piperine, both *in vitro* and *in vivo* , on cytochrome P-450 enzymes and reported that the alkaloid is a potent non-specific inhibitor of drug metabolism. Reen and Singh (1991), also confirmed the inhibitory effect of piperine on the pulmonary cytochrome P450 enzymes in rats. In the present study, longer availability of ENR could be attributed to the inhibition

of metabolism of ENR. This is also evident from the lower concentrations of CIP measured in the earlier plasma samples in piperine-treated animals as compared to normal goats. The metabolite formation rate constant was also decreased in piperine-treated goats, The C_{max} of CIP was also decreased and the t_{max} was delayed by piperine. However, the concentration of ciprofloxacin were higher in the plasma samples from 4.0 h to 24 h in piperine-treated goats.

Atal *et al.*, (1981) reported that the inhibition of metabolism by piperine is reversible and the alkaloid does not permanently damage the cytochrome P450 system. The higher ciprofloxacin concentrations in later plasma samples could be attributed to the lack of effect of piperine after a few hours. This, however, needs to be confirmed by investigating the effect of piperine on goat hepatic metabolism especially on the isozymes responsible for the metabolism of fluoroquinolones.

In this study, piperine co-treatment with ENR, produced a significant increase in bioavailability suggesting alterations in the dosage regimen of ENR.

However, caution needs to be exercised on the use of piperine in goats by subcutaneous route. Irritation at the site of injection and slight hemolysis observed in the early plasma samples call for proper standardisation before piperine could be used routinely with antimicrobial agents in domestic ruminant species.

5.5 Effect of probenecid on the plasma concentrations and pharmacokinetics of ENR and CIP in goats

Probenecid was used in this study in an attempt to enhance the bioavailability of ENR. Probenecid has already been shown to increase and sustain serum concentrations of penicillins, cephalosporins and ciprofloxacin in humans (Cunningham *et al.*, 1981).

There was a significant increase in the plasma concentrations of both enrofloxacin and ciprofloxacin in probenecid-treated goats. Probenecid co-

administration also significantly increased the AUC of ENR, suggesting a higher persistence of ENR in the body. The bioavailability was significantly increased. There were significant increase in the values of $t_{1/2}\beta$, MRT and AUC of ciprofloxacin suggesting longer persistence of the metabolite too. On the contrary, Rao (1999) did not observe any significant effect of intravenous administration of probenecid on the i.v./i.m. disposition kinetics of ENR in goats. However, in this study probenecid was administered subcutaneously. Hence a longer persistence of probenecid during the elimination phase of enrofloxacin and its metabolite ciprofloxacin could be the reason for the significant effect of probenecid. Oral administration of probenecid has also been reported to increase the bioavailability of fluoroquinolone, flumequine, in calves. Guerrini *et al.*(1985) also reported decreased renal secretion of cefotaxime in probenecid-co treated sheep after i.m. and s.c. administration but not after i.v. administration of both drugs.

When ciprofloxacin (7.5 $mg.kg^{-1}$, s.c.) was coadministered with probenecid (40 $mg.kg^{-1}$, s.c.), the plasma concentrations of ciprofloxacin were consistently higher from 10 min 12 h and the drug could be detected up to 24 h. The $t_{1/2\beta}$ and MRT were increased suggesting longer persistence of the drug. The increase in AUC and F reflect higher availability of ciprofloxacin in probenecid-treated goats which is attributed to the low clearance of ciprofloxacin in these animals. Jachde *et al.* (1995) also reported increase in values of AUC, MRT and $t_{1/2}\beta$ of CIP and its metabolite M1 in humans, who were coadministered probenecid.

The s.c. bioavailability of ciprofloxacin was very low (32%). This was appreciably enhanced to 59% in probenecid-treated goats. However, owing to the poor bioavailability of this drug by s.c. route, use of ciprofloxacin by this route may not be recommended. Nevertheless, probenecid may be used to enhance the bioavailability of ciprofloxacin administered by other routes The present study indicates the existence of a probenecid-sensitive active transport in renal tubules

for both ENR and CIP, which can be utilised for the purpose of enhancement of bioavailability of these drugs by concurrent use of probenecid.

5.6 Plasma concentrations and pharmacokinetics of ciprofloxacin after single intravenous administration (7.5 mg kg^{-1}) in goats

The plasma concentration versus time plot of ciprofloxacin showed a biphasic decline with a steep decline upto 0.25 h followed by a slow elimination phase with detectable concentrations upto 10-12 h in goats.

For characterizing the pharmacokinetics of ciprofloxacin in phases VI, VII and VIII of the study, non-compartmental analysis was used as there were difficulties in fitting the curve satisfactorily in phases VII and VIII. Non-compartmental analysis is based on statistical moments theory and is being frequently employed recently in lieu of compartmental analysis.

After intravenous administration, the AUC obtained for CIP was 11.29 $\mu g.h.ml^{-1}$ (9.63-13.52). The AUC obtained in this study is comparatively higher than the AUC obtained in sheep after the same i.v. dose. (AUC, 421.43 mg.min.L: Munoz *et al.*, 1996). In cow calves, Kumar *et al.* (1997) reported an AUC of 352.34 $\mu g.min.ml^{-1}$ following i.v. administration of ciprofloxacin (5 mg.kg^{-1}). In buffalo calves, the AUC was comparable to that of cow calves (5.56 μg h. ml^{-1}) (Raina *et al.*, 2000). In lactating cows, a much lower AUC of 198.66 $\mu g..min..ml^{-1}$ has been reported (Jaya Kumar *et al.*, 2000).

The $t_{1/2\beta}$ was calculated as a ratio of 0.693 to the elimination rate constant (β) calculated by linear regression analysis. The median $t_{1/2\beta}$ of CIP obtained in this study was 1.435 h, which is comparable to the $t_{1/2\beta}$ reported in sheep (72.3 min: Munoz *et al.*, 1996). However, much longer $t_{1/2\beta}$ of ciprofloxacin has been reported in lactating cows (129.33 min.; Jayakumar *et al.*, 2000), cow calves (194.3 min.;Kumar *et al*, 1997) and buffalo calves (3.54 h.;Raina *et al.*, 2000).

The mean residence time (MRT) obtained in the present study was 1.843 h, which is in close agreement with the value obtained in sheep (88.43 min; Munoz *et al.*, 1996) However, in buffalo calves (4.76 h; Raina *et al.*, 2000), cow calves (4.01 h; Kumar *et al.*, 1997) and lactating cows (182.2 min.; Jayakumar *et al.*, 2000) a higher values of MRT of CIP have been reported.

$V_{d\ area}$ obtained for ciprofloxacin in this study was 1.25 $L.kg^{-1}$. Munoz *et al.* (1996) reported slightly higher $V_{d\ area}$ for ciprofloxacin in sheep (1.89 $L.kg^{-1}$). However, higher values of $V_{d\ area}$ have been reported in lactating cows (2.84 $L.\ kg^{-1}$; Jayakumar *et al.*, 2000), buffalo calves (3.61 $L.kg^{-1}$; Raina *et al.*, 2000) and cow calves (4.05 $L.kg^{-1}$; Kumar *et al.*, 1997). These variations indicate that there are species differences in the disposition kinetics of ciprofloxacin. In the phase I of the present study, ENR exhibited a $V_{d\ area}$ of 0.863 $L.kg^{-1}$. It is thus apparent that ciprofloxacin may have better tissue penetration than enrofloxacin in goats. The total body clearance of CIP was 664.2 $ml\ h^{-1}.kg^{-1}$ which was also higher than the Cl_B of ENR (502.5 $ml.h^{-1}.kg^{-1}$) obtained in the phase I of this study. However, relatively higher clearance values for ciprofloxacin have been reported in buffalo calves (73.1 $ml.h^{-1}.kg^{-1}$), cow calves (14.29 $ml.min^{-1}.kg^{-1}$), lactating cows (15.1ml.$min^{-1}.kg^{-1}$) and sheep (0.018 $L.h^{-1}.kg^{-1}$) (Raina *et al.*, 2000; Kumar *et al.*, 1997; Jayakumar *et al.*, 2000 and Munoz *et al.*, 1996).

5.7. Plasma concentrations and phar macokinetics of ciprofloxacin after single subcutaneeous administration (7.5 $mg.kg^{-1}$) in goats

Ciprofloxacin was detected in plasma samples from two minutes. The peak plasma drug concentration (C_{max}) was 1.787 $\mu g\ ml^{-1}$ which occurred at 10 min. (t_{max} 0.167 h). Munoz *et al.* (1996) reported a low C_{max} of 0.69 $\mu g\ ml^{-1}$ in sheep after single i.m. administration of CIP. However, the time to peak concentration was

31.96 min which is comparable to this study. To our knowledge, s.c. route has not been employed for administration of ciprofloxacin in other animal species so as to give a comparative picture.

In comparison to ENR, which was administered at a dose of 5 mg kg^{-1} s.c. in the phase II of the study, the C_{max} of CIP was much lower (2.819 $\mu g.ml^{-1}$ for ENR). However, the t_{max} for ENR was obtained at 1 h. The MAT for CIP was 1.784 h as compared to 1.237 h for ENR which indicates longer absorption phase for CIP than ENR. The AUC obtained for CIP after single s.c. administration was 3.651 $\mu g\ h\ ml^{-1}$ with the bioavailability of 32.8%. The poor bioavailability of CIP is in contrast to the complete bioavailability of enrofloxacin after s.c. administration. The poor bioavailability for CIP may be attributed to the poor absorption of the drug from site of injection or due to the presence of a deep seated tissue compartment from which the drug is released slowly over a long period of time while the drug is rapidly cleared from the plasma.

The results of the present study indicate the futility of CIP administration by s.c. route to be used in systemic infections owing to its lower plasma concentration and poor bioavailability in goats. However, the utility of this route remains to be tested for some deep seated infections where the drug may be stored for a longer time.

5.8 Relationship between pharmacokinetic and pharmacodynamic parameters – optimisation of dosage regimens of ENR and CIP in goats

The outcome of a pharmacokinetic study is to suggest guidelines for appropriate dosage regimens of the drug in the target species. The dosage regimens recommended should be reflected in the clinical efficacy of the treatment. Recently, several relationships between pharmacokinetic parameters and measures of

antimicrobial activity have been proposed and evaluated in humans and a lot of interest has been generated on the prediction of clinical efficacy based on pharmacokinetics of a drug. Based on the results obtained in this study, an attempt has been made to suggest suitable dosage regimens of ENR and CIP for use in goats.

Fluoroquinolones, like ENR and CIP are concentration-dependent killing agents (Dudley, 1991) i.e. their efficacy depends on the higher plasma concentration of the drug and not on the amount of time the drug concentration remains above MIC. This combined with the fact that the fluoroquinolones exert PAE, could well be one of the guiding factors in the optimisation of dosage schedule.Fluoroquinolones exert a PAE of 4-8 h against a number of strains including *E.coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Brown, 1996) and PAE *in vivo* is generally longer than PAE *in vitro* due to postantibiotic sub-MIC effect (PASME) and the postantibiotic leukocyte enhancement (PALE) exerted *in vivo* (Walker, 2000).

Taking the above factors into consideration, several workers have proposed that C_{max} /MIC and AUC/MIC ratios are the best indicators for a good clinical outcome. Based on studies in *in vitro* models and clinical trials, it has been shown that maximal clinical efficacy of fluoroquinolones may be achieved when the C_{max} -to-MIC ratios are more than 8-12 and AUC-to-MIC ratio >100-125 (Walker, 2000).

Applying these principles to the pharmacokinetic parameters obtained for both ENR and CIP in the different phases of the present study and considering 0.1 $\mu g\ ml^{-1}$ as the minimum inhibitory concentration (MIC) of enrofloxacin and ciprofloxacin against majority of veterinary pathogens (Kaartinen *et al.*, 1997b), the following results were obtained (Table 41).

Table 41. Relationship between pharmacokinetic and pharmacodynamic parameters of ENR and CIP in goats

Phase of study	Treatment	Drug	Dose ($mg.kg^{-1}$)	Route	C_{max} MIC	AUC/ MIC
I	Normal	ENR	5 mg	i.v.	-	123.3[a]
II	Normal	"	5 mg	s.c.	28	83.5[a]
III	Febrile	"	5 mg	s.c.	30	125[a]
IV	Piperine	"	5 mg	s.c.	18	158[a]
V	Probenecid	"	5 mg	s.c.	25	155[a]
VI	Normal	CIP	7.5 mg	i.v.	-	113
VII	"	"	7.5 mg	s.c.	18	30
VII	Probenecid	"	7.5 mg	s.c.	19	66

[a]AUC/MIC ratio for ENR treatment groups was calculated as AUC ENR+ AUC CIP/MIC

From the above table, it is obvious that use of i.v. dosage of ENR ($5mg.kg^{-1}$) is likely to produce an ideal clinical outcome. The s.c. dosage did produce a very high C_{max} / MIC ratio. Though slightly falling short of expected AUC/MIC ratio, the s.c. route, may still be an ideal route since high C_{mac} / MIC is a better predictor of clinical efficacy.

Taking the PAE into consideration, it is recommended that ENR could be ideally used at the dose of 5 $mg.kg^{-1}$ i.v. or s.c. every 12 hourly. In febrile goats, by the same considerations, since not much of alterations were found in the pharmacokinetics, the same dosage schedule will hold good.

The use of piperine and probenecid sustained the maintenance of therapeutic concentrations beyond 12 h and enhanced the bioavailability of ENR, besides appreciably increasing the AUC/MIC ratio. With co-administration of either piperine or probenecid the dosing interval of ENR may be extended upto once in 24 h for the same dosage.

While the pharmacokinetics of CIP following i.v. administration have shown adequacy to recommend the dose of 7.5 $mg.kg^{-1}$ every 12 h, the s.c. use of ciprofloxacin cannot be recommended owing to very low AUC/MIC ratio. Probenecid could not increase the AUC/MIC ratio of CIP to the desired extent and hence co-administration of probenecid with CIP (7.5 $mg.kg^{-1}$, s.c.) cannot be recommended for clinical use in goats.

SUMMARY

In this study, the detailed pharmacokinetics of ENR and its active metabolite CIP were investigated in normal, febrile, probenecid-treated and piperine-treated goats after administration of ENR. Besides, the pharmacokinetics of CIP was also investigated in normal and probenecid-treated goats after its i.v. or s.c. administration.

In phase I of the present study, ENR was given intravenously at a dose of 5 $mg.kg^{-1}$ and the concentrations of ENR and its active metabolite CIP were determined in plasma by HPLC assay. The concentrations of ENR and CIP were detectable in plasma up to 8 and 5 h, respectively. The plasma concentration-time data of ENR could be best fitted to a two-compartment open model and that of CIP to a one-compartment open model.

The important pharmacokinetic parameters of ENR calculated were as follows : $t_{1/2\beta}$, 1.157 h, AUC, 9.95 $\mu g.h.ml^{-1}$; MRT, 1.359 h; $V_{d\,area}$, 0.863 $L.kg^{-1}$; and Cl_B,

502.5 ml. $h^{-1}.kg^{-1}$. The main pharmacokinetic parameters of CIP were : $t_{1/2\beta}$, 0.815 h; C_{max}, 1.159 $\mu g.ml^{-1}$; t_{max}, 1.5 h; and MR, 0.308.

In phase II of the study, ENR was administered at a dose of 5 $mg.kg^{-1}$ by subcutaneous route. ENR concentratons could be detected in plasma upto 10 h and that of CIP upto 7 h. The pharmacokinetics of ENR and CIP could be adequately described by a one compartment open model with first order absorption. The important pharmacokinetic parameters of ENR were : $t_{1/2\beta}$, 1.301 h; AUC, 6.58 $\mu g.h.ml^{-1}$; MRT, 2.43 h; F, 104.3%; C_{max}, 2.819 $\mu g.ml^{-1}$ and t_{max}, 1.0h. The important kinetic parameters of CIP were as follows : $t_{1/2\beta}$, 1.259 h; C_{max}, 0.709 $\mu g.ml^{-1}$; t_{max}, 1.5h and MR, 0.192.

In phase III of the study, plasma concentrations and pharmacokinetics of ENR and CIP were determined in endotoxin-induced febrile goats after s.c. administration of ENR (5 $mg.kg^{-1}$). Endotoxin induced marked pyrexia in all the goats and an increase in rectal temperature of atleast 1- 1.5°F which was maintained upto 12 h.

ENR was detected in plasma up to 12 h and CIP upto 8.0 h. The important pharmacokinetic parameters were : $t_{1/2\beta}$, 1.361 h; AUC, 10.53 $\mu g.h.ml^{-1}$, MRT, 3.075 h; F, 99.15; C_{max} 2.993 $\mu g.ml^{-1}$; t_{max}, 1.5 h. The important pharmacokinetic parameters of CIP were : $t_{1/2\beta}$, 1.222 h, AUC, 1.96 $\mu g.h.ml^{-1}$; MRT, 4.01 h and MR, 0.188.

In phase IV of the study, plasma concentrations and pharmacokinetics of ENR and CIP were determined after concurrent administration of ENR (5 $mg.kg^{-1}$, s.c.) and piperine (2 $mg.kg^{-1}$, s.c.). Detectable concentrations of ENR and CIP could be found in plasma up to 24 h. The important pharmacokinetic parameters of ENR were : $t_{1/2\beta}$, 3.012h; AUC, 12.71 $\mu g.h.ml^{-1}$; MRT, 5.973 h; C_{max}, 1.809 $\mu g.ml^{-1}$; t_{max}, 4.0 h; F and 132.9%. The important pharmacokinetic parameters of CIP were $t_{1/2\beta}$,

2.96 h; AUC, 3.09 μg.h.ml^{-1}; C_{max}, 0.440 μg ml^{-1}; t_{max}, 4.0 h and MR, 0.243. All the important pharmacokinetic parameters of ENR were significantly different in piperine-treated goats as compared to normal goats.

In phase V of the study, effect of probenecid (40 mg.kg^{-1}, s.c.) was investigated on the plasma concentrations and pharmacokinetics of ENR and CIP following concurrent administration of probenecid and ENR (5 mg.kg^{-1}, SC) in goats. In this study, plasma ENR concentrations could be detected upto 12 h and that of CIP upto 24 h. The important pharmacokinetic parameters of ENR were : $t_{1/2\beta}$, 1.848 h; AUC, 15.91 μg.h.ml^{-1}; MRT, 4.44 h; C_{max}, 2.534 μg.ml^{-1}; t_{max}, 3.0 h and F, 159.9%. Important pharmacokinetic parameters of CIP were : $t_{1/2\beta}$, 3.314 h; AUC, 264 μg.h.ml^{-1}; C_{max}, 0.315 μg.ml^{-1}; t_{max}, 3.0 h and MR, 0.177. Significant differences were observed in AUC, Cl_B and F of ENR in probenecid-treated goat as compared to normal goats. Co-administration of probenecid also significantly altered all the important pharmacokinetic parameters of CIP.

In phase VI of the study, plasma concentrations and pharmacokinetics of CIP were investigated after its single intravenous administration (7.5 mg.kg^{-1}). CIP could be detected in plasma up to 10 h. The important pharrmacokinetic parameters were : $t_{1/2\beta}$, 1.435 h; AUC, 11.29 μg.h.ml^{-1}; MRT, 1.843 h; $V_{d\,area}$, 1.258 L.kg^{-1}; Cl_B, 664.2 ml.h^{-1}.kg^{-1} and V_{dss}, 1.224 L.kg^{-1}.

In phase VII of the study, plasma concentrations and pharmacokinetics of CIP were investigated after its single subcutaneous administration (7.5 mg.kg^{-1}). The important pharmacokinetic parameters were : $t_{1/2\beta}$, 2.761 h; AUC, 3.651 μg.h.ml^{-1} MRT, 3.551 h; C_{max}, 1.787 μg.ml^{-1}; t_{max}, 0.167 h and F, 32.8%.

In phase VIII of the study, effect of probenecid (40 mg/kg^{-1}, s.c.) was investigated on the plasma concentrations and pharmacokinetics following concurrent administration of probenecid with CIP (7.5 mg.kg^{-1}, s.c.). CIP were

detected in plasma upto 24 h. The important pharmacokinetic parameters were : $t_{½\beta}$, 3.5 h; AUC, 6.661; t_{max}, 0.167 h and F, 59.0%. There were appreciable differences in AUC, MRT, F%, Cl_B, Cl_B/F and $V_{d\,area}$ in probenecid treated goats when compared with normal goats.

The following conclusions were drawn from the present study :

1. Pharmacokinetics of ENR conformed to a two-compartment open model after i.v. administration. In goats, ENR was metabolised to CIP to the extent of 30.8%. Goats tend to eleminate ENR and CIP faster than other ruminant species.

2. After s.c. administration of ENR (5 $mg.kg^{-1}$), the drug was rapidly absorbed and conformed to a one compartment open model. The bioavailability of ENR was $\simeq$ 100% and CIP was formed to the extent of 19.3%. In view of the excellent systemic availability, ENR may preferably be administered by subcutaneous route in goats.

3. Febrile state produced moderate changes in the pharmacokinetics of ENR and CIP, with a slightly longer persistence of the drug in the plasma and these alterations may not necessitate the recommendation of any modification in dosage regimens of enrofloxacin.

4. Piperine co-administration with ENR, produced longer persistence of the drug in the plasma and significant changes in the pharmacokinetics of ENR. Owing to its ability to cause irritation and mild hemolysis, the use of piperine needs to be standardised in goats.

5. CIP, when injected i.v., produced detectable concentrations in the plasma up to 10 h, and the pharmacokinetic parameters were comparable to that of ENR.

6. After s.c. administration, the bioavailability of CIP was relatively poor indicating that subcutaneous route may not be appropriate for administration of CIP in goats.

7. Co-administration of probenecid increased the persistence of ENR and CIP in the plasma and decreased their systemic clearance. Coadministration of probenecid with CIP, though increased the drug bioavailability by 100%, the administration of CIP by s.c. route may not be recommended in goats due to its overall inadequate bioavailability.

8. Based on the pharmacokinetic predictors of clinical efficacy, i.e., C_{max}/MIC ratio and AUC/MIC ratio (taking 0.1 $\mu g.ml^{-1}$ as the MIC for majority of veterinary pathogens), ENR may be administered subcutaneously at 5 $mg.kg^{-1}$ every 12 h for the treatment of drug susceptible infections in goats. ENR (5 $mg.kg^{-1}$, s.c.) may be administered once daily when the drug is co-administered with either piperine or probenecid. A suitable intravenous dosage regimen of CIP for the treatment of drug-susceptible infections in goats would be 7.5 $mg.kg^{-1}$ every 12 h.

MINI ABSTRACT

Enrofloxacin (ENR) is a fluoroquinolone antimicrobial approved exclusively for veterinary use. Ciprofloxacin (CIP), another fluoroquinolone is a popular drug in human medicine. ENR is converted to CIP *in vivo*. In this study, pharmacokinetics of ENR and its metabolite CIP were investigated after i.v. or s.c. administration of ENR in normal, febrile, piperine-treated and probenecid-treated goats. Besides, the pharmacokinetics of CIP was also studied after i.v. or s.c. administration in normal and probenecid-treated goats.

In all groups, blood samples were collected at predetermined time intervals after drug administration and concentrations of ENR and/or CIP in plasma were assayed by a HPLC method.

After i.v. administration of ENR (5 $mg.kg^{-1}$), the important pharmacokinetic parameters were : $t_{1/2\beta}$, 1.157 h; AUC, 9.95 $\mu g.h.ml^{-1}$; Cl_B, 502.5 $ml.h^{-1}.kg^{-1}$; and $V_{d_{area}}$, 0.863 $L.kg^{-1}$. ENR was converted to CIP to the extent of 30.8%. After s.c. administration of ENR (5 $mg.kg^{-1}$) the pharmacokinetic parameters were : $t_{1/2\beta}$, 1.301h; AUC, 6.58 $\mu g.h.ml^{-1}$, C_{max}, 2.819 $\mu g\, ml^{-1}$, t_{max}, 1.0 h and F, 104.3%.

The pharmacokinetics of ENR was not significantly altered in febrile goats to merit any changes in dosage regimen. Co-administration of piperine (2$mg.kg^{-1}$.s.c.) or probenecid (40 $mg.kg^{-1}$, s.c.) significantly increased the persistence and bioavailability of ENR in goats. When CIP was administered i.v. to goats at a dose of 7.5 $mg.kg^{-1}$, the important pharmacokinetic parameters were : $t_{1/2\beta}$, 1.435 h; AUC, 11.29 $\mu g.h.ml^{-1}$; MRT 1.843 h; Cl_B 664.2 $ml.h^{-1}.kg^{-1}$ and $V_{d_{area}}$ 1.258 $L.kg^{-1}$. After s.c. administration of CIP (7.5 $mg.kg^{-1}$) the pharmacokinetic parameters of CIP were : $t_{1/2\beta}$, 2.701 h, AUC, 3.657 $\mu g.h.ml^{-1}$. C_{max} 1.787 $\mu g.ml^{-1}$; t_{max} 0.167 h and F 32.8%. The coadministration of probenecid enhanced the bioavailability of CIP by nearly two times (from 32.8% to 59%) in goats.

Based on the calculated pharmacokinetic predictors of clinical efficacy such as C_{max}/MIC ratio and the AUC/MIC ratio, an optimal subcutaneous dosage regimen of ENR for treatment of drug susceptible infections in goats would be 5 $mg.kg^{-1}$, repeated at 12 h interval. When ENR is co-administered with either piperine or probenecid, the dosing interval of ENR may be reduced to once daily.

CIP may also be recommended for use in goats, with a dosage schedule of 7.5 $mg.kg^{-1}$ i.v., twice daily. Due to its poor bioavailability s.c. administration of CIP may not be recommended in goats.

लघु सारांश

एनरोफ्लाक्ससिन और सिप्रोफ्लाक्सासिन पशु चिकित्सा में बहुत उपयोगी प्रतिसूक्ष्म जीवी कारक है । वर्तमान अन्वेषण में एनरोफलाक्सासिन की रक्तजल में मात्रा एवं विस्तृत फार्मोकोकाईनेटिक्स का अज में, अंर्तविष प्रेरित सज्वर अज में, पैपरिन–अभिक्रियत और प्रोबेनिसेड–अभिक्रियत अज में अध्ययन किया गया । सिप्रोफ्लाक्सासिन की भी रक्तजल में मात्रा एवं विस्तृत फार्मोकोकाईनेटिक्स का अज में और प्रोबेनिसेड–अभिक्रियत अज में गहन अध्ययन किया गया । रक्तजल में दोनो दवा की मात्रा निर्धारण के लिए एच.पी.एल.सी. अमापन उपयोग किया गया ।

एनरोफ्लाक्सासिन 5 मि.ग्रा. प्रति कि.ग्रा. मात्रा से अज में अंतशिरारीय व अवत्वकीय दिये जाने पर क्रमानुसार दवा की इष्टतम चिकित्सीय सांद्रता 6 और 7 घंटे तक रक्तजल में पाई गयी । अवत्वकीय दिये जाने पर इस दवा का संपूर्ण रक्तजल में लाभ हुआ और पूर्णतम वितरण की प्राप्ति हुई । अज में लगभग 30 प्रतिशत तक एनरोफ्लाक्सासिन का सिप्रोफ्लाक्सासिन में रूपांतर हो गया । अज में एनरोफ्लाक्सासिन का अर्ध आयु और निष्काषन दर 1.157 घटा और 503 मि.ली. प्रति घंटा प्रति कि.ग्रा. पाये गये । सज्वर अज में दवा की सांद्रता 8 घंटे तक पैपरिन–अभिक्रियत और प्रोबेनिसेड–अभिक्रियत अज में 12 घंटे तक पाई गयी । विशेष रूप से सज्वर अज में उपपचाय कम होने के कारण सिप्रोफ्लाक्सासिन की मात्रा कम हो गयी । प्रोबेनिसेड–अभिक्रियत अज में उपपचाय की मात्रा अधिक पाई गयी और एनरोफ्लाक्सासिन और उनका उपपचाय सिप्रोफ्लाक्सासिन की फार्मोकोकाईनेटिक प्राचलिया में अतर पडा । सिप्रोफ्लाक्सासिन 7.5 मि.ग्रा. प्रति कि.ग्रा. मात्रा से अज में अंतशिरारीय व अवत्वकीय दिये जाने पर दवा की इष्टतम चिकित्सीय सांद्रता 8 घंटे तक पाई गयी और प्रोबेनिसेड–अभिक्रियत अज में 10 घंटे तक पाई गयी । अवत्वकीय दिये जाने पर सिप्रोफ्लाक्सासिन का केवल 32 प्रतिशत तक वितरण की प्राप्ति हुई । वर्तमान शोध के परिणाम से अज में एनरोफ्लाक्सासिन 5 मि.ग्रा. प्रति 12 घंटे अवत्वकीय दे सकते हैं । सज्वर की स्थिति में इस मात्रा निर्धारण के परिवर्तन की आवश्यकता नहीं है । सिप्रोफ्लाक्ससिन 7.5 मि.ग्रा. प्रति 12 घंटे अंतशिरारीय दे सकते हैं । पैपरिन और प्रोबेनिसेड को औपयोगिता का मूल्यांकन की जरूरत है ।

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VITA

Dr. S. Ramesh, completed his B.V.Sc. in 1989 and M.V.Sc. (Pharmacology) in 1992 from Madras Veterinary College. He served as Veterinary Assistant Surgeon for 2½ years before joining as Assistant Professor in Tamilnadu Veterinary and Animal Sciences University in 1994.