Pharmacokinetics of Enrofloxacin And its Interaction with Diclofenac in Buffalo Calves

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

BIHAR

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

PHARMACOLOGY & TOXICOLOGY

By

Nitesh Kumar

Registration No.- M/V. P. T./21/1999-2000

Department of Pharmacology & Toxicology

BIHAR VETERINRAY COLLEGE

PATNA - 800 014

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2002

Dedicated to

My Parents

&

Teachers

Dr. C. Jayachandran
Ph. D.
Associate Professor
Department of Pharmacology & Toxicology
Bihar Veterinary College,
Patna – 14 (India)

CERTIFICATE – I

This is to certify that the thesis entitled **"PHARMACOKINETICS OF ENROFLOXACIN AND ITS INTERACTION WITH DICLOFENAC IN BUFFALO CALVES"** submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Veterinary Pharmacology & Toxicology) of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa is the record of bonafide research carried out by **Dr. Nitesh Kumar** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

Jayachandran 9/2/02

(C. Jayachandran)
Major Advisor

Endorsed :

09/02/02

(Chairman / Head of the Department)

CERTIFICATE – II

We, the undersigned members of the Advisory Committee of **Dr. Nitesh Kumar**, a candidate for the degree of Master of Veterinary Science with Major in Veterinary Pharmacology & Toxicology, have gone through the manuscript of the thesis and agree that the thesis entitled "**PHARMACOKINETICS OF ENROFLOXACIN AND ITS INTERACTION WITH DICLOFENAC IN BUFFALO CALVES**" may be submitted by **Dr. Nitesh Kumar** in partial fulfilment of the requirements for the degree.

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CERTIFICATE – III

This is to certify that the thesis entitled **"PHARMACOKINETICS OF ENROFLOXACIN AND ITS INTERACTION WITH DICLOFENAC IN BUFFALO CALVES"** submitted by **Dr. Nitesh Kumar** in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Veterinary Pharmacology & Toxicology) of the faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, Pusa was examined and approved on ...14.5..... 2002.

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9/2/02

(Nitesh Kumar)

Patna

Date : 09/02/02.

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Chapter – 1

Introduction

INTRODUCTION

Over the last decade, it has become increasingly clear that the effects of many drugs, when given concurrently, are not necessarily predictable on the basis of knowledge of their effects when given alone. The subject of drug interactions interests pharmacologists, and it is now highly important to clinical practitioners. Although the original observations about such interactions stemmed from fundamental research, subsequent knowledge of drug interactions, acquired from experiments on animals, has been used to therapeutic advantages in animals and man or enables a physician to minimize or prevent drug toxicity by adjustment of the dosage.

Antimicrobial agents play a major role in medical and veterinary practices in combating various systemic microbial infections. Systemic microbial infections generally cause pyrexia and/or inflammation associated with pain and hence non-steroidal anti-inflammatory drugs (NSAIDs) are usually administered along with antimicrobials. Quinolones are remarkably free from toxicity in animals. The fluoroquinolones are a class of antimicrobials that are frequently used in veterinary practices to treat a variety of infections (Greene and Budsberg, 1973). They posses broad spectrum with bactericidal activities (Wolfson and Hooper, 1985; Vancutsem *et al.*, 1990).

Enrofloxacin, a recent fluoroquinolone carboxylic acid derivative, developed exclusively for veterinary use (Altreuther, 1987; Chu and Fernandes, 1989). It possesses a broad-spectrum activity against gram-negative bacteria such as *Escherichia coli*, *Salmonella*, *Klebsiella*, *Proteus*, *Haemophilus*, *Pasteurella*, *Campylobacter*, *Pseudomonas* sps. (Scheer, 1987) and also against gram positive bacteria as well as *Mycoplasma* (Bauditz, 1990). Enrofloxacin effectively penetrates all organs and tissues and the distribution pattern is more or less similar in all species. In man and animals, enrofloxacin is de-ethylated to ciprofloxacin (Tyczkowska *et al.*, 1989 ; Flammer *et al.*, 1991) which is a potent antimicrobial agent used in human medicine (Bergan *et al.*, 1988). Both enrofloxacin and ciprofloxacin are bactericidal at very low concentrations for a broad spectrum of gram-negative and gram-positive bacteria and *Mycoplasma* (Hooper & Wolfson, 1991). Enrofloxacin is suitable for the treatment of septicaemia, gonorrhaea, respiratory infections, urinary tract infections, skin infections as well as soft tissue, bone and joint infections. It is also active against several organism which are resistant to many other antimicrobials.

Antimicrobials and non-steroidal anti-inflammatory, analgesic and antipyretic agents are frequently used concomitantly and pharmacokinetic interactions between them have been described (Kampmann *et al.*, 1972 ; Carbon *et al.*, 1981, 1984). In experimental staphylococcal osteomyelitis, ibuprofen given concomitantly with

oxacillin significantly increased antibiotic efficacy but the mechanism of interaction was not studied (Khurana and Deddish, 1986). Joly *et al.* (1988) showed enhancement of the therapeutic effects of cephalosporins (cefotiam, cefmenoxime and ceftriaxone) in experimental endocarditis by altering their pharmacokinetics when simultaneously used with the non-steroidal anti-inflammatory drug diclofenac. No effect of diclofenac on the pharmacokinetics of cloxacillin was shown in man by Nergelius *et al.* (1997). Surya Kumar *et al.* (1995) showed rifampicin pretreatment reduces bioavailability of diclofenac sodium. Influence of enrofloxacin on theophylline steady-state pharmacokinetics in the Beagle dog was demonstrated by Intorre *et al.* (1995).

Diclofenac is a potent NSAID as well as analgesic compound with good antipyretic and uricosuric properties (Maier *et al.*, 1979). It produces its effects by irreversibly inhibiting the cyclooxygenase pathway of prostaglandin synthesis, which is the most common mediator of pain, inflammation and pyrexia in man and animals. It is used in degenerative joint diseases, rheumatoid arthritis, ankylosing spondylitis and allied conditions (Brodgen *et al.*, 1980).

In India, buffalo rearing is most popular among farmers. Buffalo is the chief milk yielding animal in Indian subcontinent, which contribute to the upliftment of the poor farmers of this country. Keeping in view the major contribution of buffaloes in

national economy and huge employment avenues, its proper and effective health coverage is very much essential by achieving the new dimension through enrofloxacin and diclofenac combination therapy.

For judicious use of an antimicrobial and a NSAID, a rational dosage regimen is a pre-requisite for which detailed pharmacokinetic study is needed. In order to use drugs effectively, it is important to investigate the detailed pharmacokinetics of the drug in the same species and also in similar climate in which the drug is to be used clinically (Nawaz *et al.*, 1980). Pharmacokinetic studies of antimicrobials and NSAIDs are carried out in healthy animals to obtain detailed pharmacokinetic data. From these data, appropriate dosage regimen is derived for the effective treatment of the disease, when drug administered alone. Now, it is well established that the kinetic parameters of a drug may differ during combination therapy resulting into sharp change in dosage regimen.

Pharmacokinetic studies on enrofloxacin were carried out in different species of animals though little work has been done on buffalo calf, particularly on the interaction of diclofenac with enrofloxacin. Keeping in view of the aforesaid facts, the present investigation was carried out in buffalo calves with the following specific aims and objectives :

1. Estimation of concentrations of enrofloxacin and diclofenac at different time intervals in body fluids following i.v. administration when given alone in buffalo calf.

2. Determination of kinetic parameters of enrofloxacin and diclofenac when given alone.

3. Calculation of dosage regimen of enrofloxacin when administered alone.

4. Estimation of concentrations in biological fluids, calculation of kinetic parameters of enrofloxacin & diclofenac and calculation of dosage regimen of enrofloxacin when the drugs are given together to know the interaction of the drugs when injected by i.v. route.

The findings of this investigation would help in a long way in making the recommendation of appropriate combination therapy of enrofloxacin with diclofenac for the effective treatment of various bacterial infections as well as to treat various inflammatory conditions in buffalo calf.

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Chapter - II

Review of Literature

REVIEW OF LITERATURE

Quinolones, the synthetic antimicrobial agents belonging to Carboxylic acid derivatives are becoming more popular in medical and veterinary practices. Initially, nalidixic acid was introduced in clinical practice in 1963. Nalidixic acid possesses narrow spectrum of activity (mostly gram negative organisms) and mainly used for treating urinary tract infections caused by gram negative organisms. Due to narrow spectrum of activity and rapid development of resistance of nalidixic acid, systematic search was carried out to synthesise agents possessing wide spectrum of antimicrobial activity which may also be used for systemic use.

Introduction of 6-fluorine atom into the basic nucleus of quinolones in fluoroquinolones produced racemic mixture in which one isomer was more active than the other which possess extended gram positive activity. Further, advancement in the quinolone field came with the synthesis of norfloxacin which because of its 6-fluorine and 7-piperazine group, had enhanced antibacterial activity. Similarly, a number of other newer fluoroquinolones have been synthesised viz. enrofloxacin, ofloxacin, ciprofloxacin, pefloxacin etc. (Harold, 1987) and some of them are effectively used in veterinary practice also for the treatment of various bacterial infections (Goldstein and Citron, 1993).

General Pharmacokinetics of Fluoroquinolones :

The general pharmacokinetic characteristic of fluoro-quinolones includes :

1. Complete parental absorption (nearly 100% bio-availability), except norfloxacin (70-90%).
2. Good tissue distribution.
3. Renal excretion accompanied by tubular secretion (responsible for drug recycling).
4. Enterohepatic recycling (proposed).
5. Hepatic metabolism via oxidation and glucuronidation.
6. Oral bio-availability is 30-90% in chicken, turkey and pigs.
7. Food, fat and high calcium diet inhibit fluoroquinolone absorption except in enrofloxacin and ciprofloxacin (Gyrd Hausen and Nielson, 1994; and Frost *et al.*, 1989).

The distribution of quinolones is very high in the body tissues owing to lower plasma protein binding, particularly in newer fluoroquinolones (Nalidixic acid > 90%, where as ciprofloxacin is 20-22%). Enrofloxacin concentrations that were upto 3 times higher than

serum concentration were observed in tissue homogenates in calves, with the following order of organs liver > kidney > heart > lung > spleen > intestinal wall > serum = muscles (Scheer, 1987).

Penetration into the CNS is relatively good and vitreous humor penetration is approximately 20% (Barza, 1991), and 29% of the serum activity in cortical bones (Duval and Budsberg, 1995). There is about 16 times more placental transfer of enrofloxacin than ciprofloxacin showing a specific transport mechanism to the foetus (Aramayona *et al.*, 1994).

Apart from nasal secretions and ejaculate, body fluids concentration of fluoroquinolones rarely reach the plasma concentration (Sorgel *et al.*, 1989). Thus, the higher tissue concentrations are the result of sequestration (excretion) onto, or within the cells or cellular components of the tissues. For example, the intracellular concentration of fluoroquinolones in polymorphonuclear leukocytes is about 7-14 times than those found in the extracellular fluid (Zweerink and Edison, 1988).

The metabolism of the fluoroquinolones is highly variable but can be extensive. The primary mechanism of metabolism and metabolites produced from commonly used fluoroquinolones are shown below :

Mechanism	Metabolites Produced
N - dealkylation	Enrofloxacin → Ciprofloxacin
N- dealkylation	Pefloxacin → Norfloxacin
Oxidation of piperazine ring	Norfloxacin → Oxonorfloxacin
Hydroxylation	Nalidixic acid → Hydroxynaladixic acid
Glucuronidation	Norfloxacin → Norfloxacin glucuronide
Sulfoxidation	Ciprofloxacin → Sulfociprofloxacin
Acetylation	Norfloxacin → N-acetylnorfloxacin

Source : Brown (1996).

After an oral dose, more than 90% of the drug is excreted in faeces. Enterohepatic circulation of the drug has also been reported (Nix and Schentag, 1988). Ramon *et al.* (1994) have indicated the elimination of ciprofloxacin by active transepithelial elimination into the bowel lumen also. Unbound fluoroquinolone molecules are filtered in the glomerulus of the kidney. Active tubular secretion is also known to occur by the organic anion transport system. Renal excretion accounts for 60% of ciprofloxacin in many species and 30-40% of norfloxacin and enrofloxacin in 24 h.

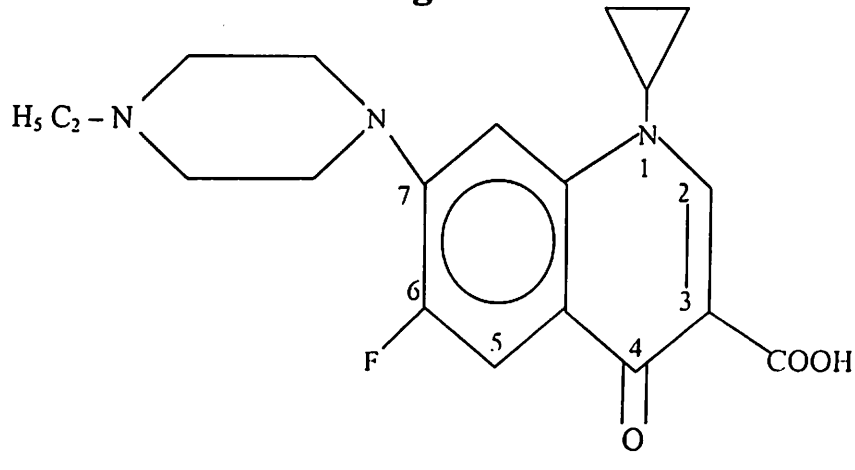
ENROFLOXACIN

Enrofloxacin, one of the newly developed fluoroquinolones was synthesised in 1983 by Bayer Research Laboratory in Germany. It is exclusively used as a drug of choice for animal treatment only. Apart from its wide spectrum of antimicrobial activity, enrofloxacin possesses excellent distribution in different tissues and body fluids. Further, it has the additional benefit of being metabolised in liver to ciprofloxacin, which also exerts potential antimicrobial activity. Renal excretion is the major route of elimination and the drug is excreted via both filtration and tubular secretion (Hooper and Wolfson, 1991).

1. Chemistry :

Enrofloxacin is a crystalline, yellowish powder with a slight bitter taste. It dissolves in either highly acidic or highly alkaline medium. The chemical structure of enrofloxacin is shown in Fig.-I.

Fig. I



1- cyclopropyl - 7 - (4-ethyl - 1- piperazinyl) - 6 - fluoro - 1, 4- dihydro - 4 - oxo - 3 - quinoline carboxylic acid.

Empirical formula - $C_{19}H_{22}FN_2O_3$ Molecular Weight - 359.40.

2. *Antimicrobial Activity :*

Enrofloxacin is a broad spectrum, antimicrobial with bactericidal action. It is effective against both gram-negative and gram positive bacteria as well as *Mycoplasmas*. In addition, some of the anaerobic pathogens are also susceptible. Development of resistance is low with other quinolone drugs. Hence, it is effective against micro-organisms that are resistance to β - lactam antibiotics, tetracyclines, aminoglycosides or macrolides and has a special place in the therapy of multi drug resistant infections. The M.I.C. values of enrofloxacin for different species of microorganism range between 0.01 to 2.0 $\mu g.ml^{-1}$ in veterinary practice.

The M.I.C. of enrofloxacin and its primary metabolite ciprofloxacin that may inhibit 90% (MIC_{90}) of veterinary clinical isolates for several species are shown below :

Organisms	**MIC_{90} Enrofloxacin ($\mu g.ml^{-1}$)**	**MIC_{90} Ciprofloxacin ($\mu g.ml^{-1}$)**
*A. pleuropneumoniae**	0.015	0.007
*A. suis**	0.015	0.001
A. pyogens	1.0	1.0
*C. pseudotuberculosis**	0.125	0.06
*E. rhusiopathiae**	0.06	0.03
H. parasuis	0.001	0.001
H. somnus	0.015	0.015

Organisms	MIC_{90} Enrofloxacin ($\mu g.ml^{-1}$)	MIC_{90} Ciprofloxacin ($\mu g.ml^{-1}$)
*P. haemolytica**	0.015	0.007
*P. multocida**	0.015	0.007
R. equi	1.0	1.0
S. equi	1.0	1.0
S. suis	1.0	1.0
S. zooepidemicus	1.0	1.0

* Organisms more susceptible to ciprofloxacin than to enrofloxacin.

Source : Prescott and Yielding (1990).

3. *Mode of Action :*

The mode of action of enrofloxacin is similar to that of other fluoroquinolones. Enrofloxacin is a bactericidal agents. The primary target site for bactericidal action of all fluoroquinolones is the "DNA gyrase" enzyme in protein synthesis. These drugs specifically inhibit the A subunit DNA gyrase, a bacterial type - II topoisomerase (Vancutsem *et al.*, 1990). Enrofloxacin penetrates the cell nucleus of bacteria and acts by inducing irreversible inhibition of DNA gyrase, a bacterial enzyme responsible for vital function of bacteria. The inhibition of gyrase by enrofloxacin stops the replication and supercoiling of DNA within a very short time and there by kills the bacteria (Crumplin *et al.*, 1984).

4. *Biochemical metabolism :*

Fig. II

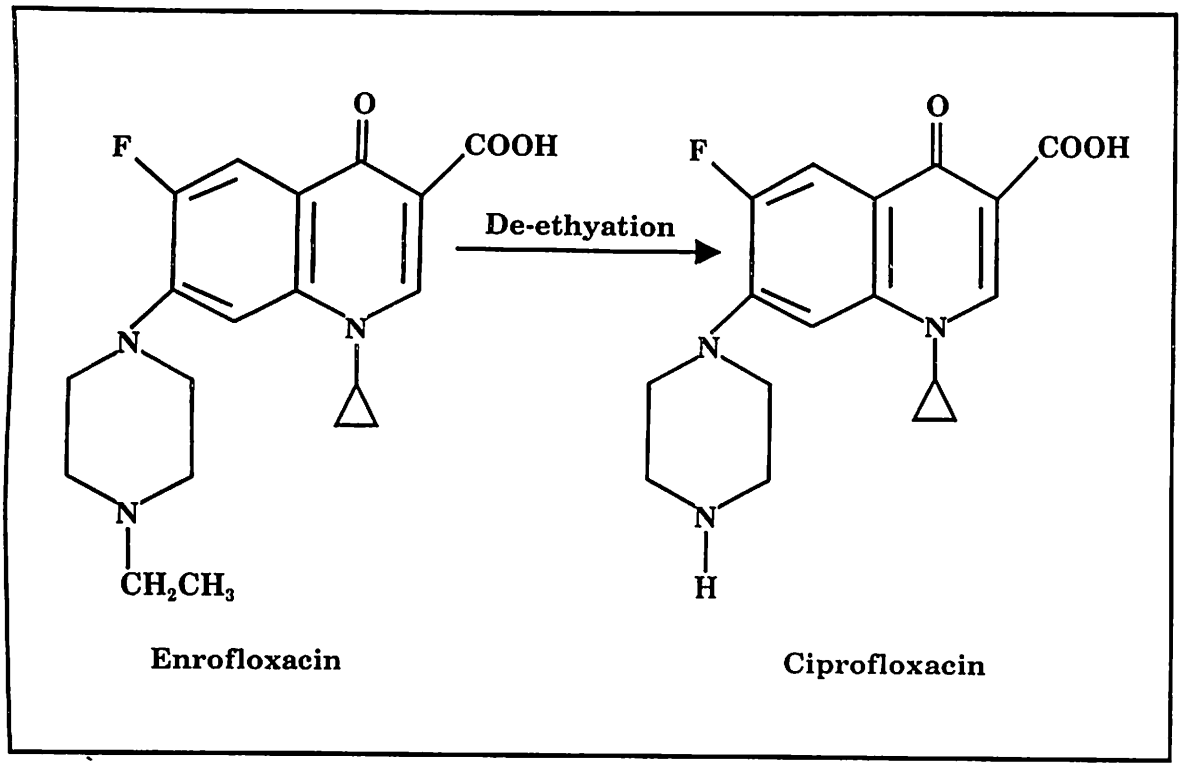


Fig.-II.-*Structural formulae of enrofloxacin and its major metabolite ciprofloxacin.*

Enrofloxacin is rapidly and widely distributed in the body tissues following administration. On metabolism, the drug is partially converted to ciprofloxacin. This metabolite has its own therapeutic value, and has been found to have a lower MIC requirement against certain gram negative microorganisms than enrofloxacin. Besides, ciprofloxacin is active against *Mycobacterium* sps. against which enrofloxacin does not have its own activity. Thus, a large spectrum of organisms are covered following the administration of enrofloxacin.

5. Pharmacokinetic Studies :

Enrofloxacin, a member of fluoroquinolone, has been exclusively introduced in animal practice. Pharmacokinetic studies of this drug were carried out in different species of animals though little work has been carried out in buffalo calf so far. Literature on kinetics of enrofloxacin in various species are stated below :

Cow :

Pharmacokinetic properties of enrofloxacin and its active metabolite ciprofloxacin were studied in five cows post i.v., i.m. and s.c. (5 mg. kg^{-1}) administration. After i.v. administration, the mean elimination half-lives of enrofloxacin and ciprofloxacin were 44 and 56 minutes, respectively. Extravascular administration was associated with delayed absorption and extended elimination half-lives (352-457 minutes). The values of volume of distribution for enrofloxacin was 0.6 L. kg^{-1}. The maximum concentration in serum after i.m. injection was 0.70 $\mu g.ml^{-1}$ and 0.14 $\mu g.ml^{-1}$ for enrofloxacin and ciprofloxacin, respectively. After i.v. injection, C_{max} for enrofloxacin in milk was 1.3 to 2.1 $\mu g.ml^{-1}$ and for ciprofloxacin 0.8 to 1.2 $\mu g.ml^{-1}$. The maximum concentration of the drug in milk was achieved 3.3 to 8h after injection (Gardorfer, 1991).

Gardorfer (1991) also reported that enrofloxacin by i.v., i.m. and s.c. routes at the dose rate of 2.5 and 5 $mg.kg^{-1}$ in cows persisted in milk three times as long as in serum. S.C. injection

produced half life of 10 to 18 h in milk, which is 2 to 4 times longer than i.v. injection. Bacteriostatic concentration in milk lasted up to 36 h.

Tras *et al.* (1993) noted the mean enrofloxacin concentration in milk samples of dairy cow after i.m. injection of enrofloxacin (2.5 $mg.kg^{-1}$) to be 0.035 ± 0.005, 0.025 ± 0.009 and 0.005 ± 0.003 $\mu g.ml^{-1}$ at 24, 48 and 72 h, respectively. They also noted that enrofloxacin could not be detected at 96 and 120 h.

Walser *et al.* (1993) conducted kinetic study of enrofloxacin after i.v., i.m. and s.c. administration of 2.5 $mg.kg^{-1}$ body weight. They noted that enrofloxacin penetrates the blood-milk barrier easily and concentrations of the drug in milk were much higher and persisted longer as compared to that of blood.

Kaartinen *et al.* (1994) estimated elimination half life of 44 minutes after i.v. administration (5 $mg.kg^{-1}$). After extravascular administration (i.m./s.c.) at the dose rate of 5 $mg.kg^{-1}$, they noted delayed absorption and extended elimination half life (350-457 minutes). Apparent volume of distribution was noted to be 0.6 $L.kg^{-1}$.

Kaartinen *et al.* (1995) noted elimination half life of 1.7, 5.9 and 5.6 h after i.v., i.m. and s.c. administration of enrofloxacin (5 $mg.kg^{-1}$). Mean absorption times were 6.2 and 6.9 h after i.m. and s.c. administration. The bioavailability after i.m. administration was 82% and 137% after s.c. administration. They noted volume of

distribution over 1 $L.kg^{-1}$ for enrofloxacin. After i.v. injection, the peak concentration of enrofloxacin in milk was reached between 0.7 and 1.3 h. After i.m. and s.c. administration, the concentration time curves for enrofloxacin in milk were shallow and there were no obvious peaks.

The pharmacokinetic behaviour of enrofloxacin was compared in four one-day-old and four one-week-old calves after i.v. administration of 2.5 $mg.kg^{-1}$ body weight by Kaartinen *et al.* (1997). They noted that mean volume of distribution at steady state (Vd_{SS}) and total body clearance (Cl_B) were significantly smaller in newborn calves. Vd_{SS} was 1.8 and 2.3 $L.kg^{-1}$, while clearance was 0.19 and 0.39 $L.kg^{-1}.h^{-1}$ in new born and one-week-old calves, respectively. Mean elimination half-life ($t_{½}$ β) did not differ significantly in newborn and in one-week-old calves ; mean $t_{½}$ β was 6.6 h and 4.9 h, respectively. They concluded that the dosage of enrofloxacin should be adjusted according to age when administered to very young calves.

McKellar *et al.* (1999) conducted kinetic study of enrofloxacin (2.5 $mg.kg^{-1}$) and danofloxacin (1.25 $mg.kg^{-1}$) after s.c. administration in ruminating calves. Mean maximum concentrations (C_{max}) ± standard deviations of enrofloxacin (0.24 ± 0.08 $\mu g.ml^{-1}$), ciprofloxacin (0.11 ± 0.03 [Total, 0.34 ± 0.10] $\mu g. ml^{-1}$) and danofloxacin (0.23 ± 0.05 $\mu g.ml^{-1}$) were detected in the plasma of calves by HPLC. The C_{max} were 0.49 ± 0.17 $\mu g.ml^{-1}$ (enrofloxacin

equivalents) and 0.24 ± 0.03 $\mu g.ml^{-1}$ (danofloxacin) when they were measured by microbiological assay. Mean C_{max} in inflammatory exudate (HPLC) were 0.18 ± 0.07 $\mu g.ml^{-1}$ (enrofloxacin), 0.10 ± 0.04 $\mu g.ml^{-1}$ (Ciprofloxacin), 0.27 ± 0.09 $\mu g.ml^{-1}$ (enrofloxacin plus ciprofloxacin) and 0.19 ± 0.05 $\mu g.ml^{-1}$ (danofloxacin) and concentration in exudate exceeded those in plasma from 8 h (enrofloxacin and ciprofloxacin) or 6 h (danofloxacin) after drug administration. The C_{max} were 0.34 ± 0.09 $\mu g.ml^{-1}$ (enrofloxacin equivalent) and 0.22 ± 0.04 $\mu g.ml^{-1}$ (danofloxacin) in exudate when they were measured by microbiological assay.

Buffalo :

Luna *et al.* (1991) administered enrofloxacin by uterine infusion (3 $mg.kg^{-1}$) and noted rapid absorption through uterine mucosa and rapidly excreted in the milk within 12 h.

Amorena *et al.* (1992) conducted kinetic study of enrofloxacin after i.v. and s.c. administration (2.5 $mg.kg^{-1}$) in six buffaloes. After i.v. administration, the initial concentration was 1.756±0.346 $\mu g.ml^{-1}$. After s.c. administration, maximum concentration of 0.20 ± 0.037 $\mu g.\ ml^{-1}$ was obtained after 70 minutes. The elimination half-life values were similar for both routes. They proposed a dose rate of 2.5 $mg.kg^{-1}$ to be repeated after every 8 h intervals.

Gatne *et al.* (1997) administered enrofloxacin by intramuscular route at the rate of 2.5 $mg.kg^{-1}$ body weight. They found a variation in the persistence of enrofloxacin in the serum, between 6 to 8 h. They have also suggested that the dose of enrofloxacin should be repeated every 12 h and in acute cases, every 8 h.

Verma *et al.* (1999) investigated the disposition kinetics and dosage regimen of enrofloxacin in breeding buffalo bulls following a single i.m. injection of 5 $mg.kg^{-1}$. The absorption half life, half-life of the terminal phase, apparent volume of distribution and total body clearance were 0.262 ± 0.099 h, 1.97 ± 0.23 h, 0.61 ± 0.13 $L.kg^{-1}$ and 210.2 ± 18.6 $ml.kg^{-1}.h^{-1}$, respectively. Therapeutic plasma levels (≥ 1 $\mu g.ml^{-1}$) were maintained for upto 6 h. A satisfactory intramuscular dosage regimen for enrofloxacin in buffalo bulls would be 8.5 $mg.kg^{-1}$ followed by 8.0 $mg.kg^{-1}$ at 8 h intervals.

Horse :

The pharmacokinetics of enrofloxacin was determined in horse through i.v. route with 5 $mg.kg^{-1}$ at 24 h interval in 5 horses (Zehe, 1990). The average half-life of 6.5 h and volume of distribution 2 $L.kg^{-1}$ with the peak concentration of 9.4 $\mu g.\ ml^{-1}$ after 10 minutes, falling to 1.9$\mu g.ml^{-1}$ after 24 h. After oral administration of 25 percent aqueous solution to 5 horses at 5 $mg.kg^{-1}$, 65 percent was absorbed when given at feeding time. An initial i.v. injection was recommended,

continued by oral administration to provide a constant serum concentration around 0.5 $\mu g.ml^{-1}$.

Giguere *et al.* (1996) noted mean distribution half life of 0.68 and 0.63 h and elimination half life of 5.94 and 6.09 h for the post i.v. doses of 2.5 and 5 $mg.kg^{-1}$ body weight, respectively. The rate constant of drug transfer from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central compartment (Kel) were noted to be 0.45 ± 0.26, 0.45 ± 0.62 & 0.32 ± 0.08 and 0.54 ± 0.55, 0.38 ± 0.10 & 0.22 ± 0.04 h^{-1} for i.v. doses of 2.5 and 5 $mg.kg^{-1}$, respectively. The apparent volume distribution of 1.22 ± 0.07 and 0.77±0.11 $L.kg^{-1}$, respectively, for i.v. doses of 2.5 and 5 $mg.kg^{-1}$. The total body clearance (Cl_B) values of 0.14 ± 0.01 and 0.09 ± 0.01 $L.kg^{-1}.h^{-1}$ for i.v. doses of 2.5 and 5 $mg.kg^{-1}$, respectively. After intragastric administration, the bioavailability was noted to be 57.39 ± 8.45 and 62.52 ± 19.65% for the dosage of 2.5 and 5 $mg.kg^{-1}$, respectively. The above workers suggested a single daily i.v. dose of 5.5 $mg.kg^{-1}$ or orally administered doses of 7.5 $mg.kg^{-1}$ every 24 h or 4 $mg.kg^{-1}$ every 12 h would be effective in horses.

The pharmacokinetic behaviour of enrofloxacin was studied in 6 horses after i.v. or i.m. administration of 5 $mg.kg^{-1}$ body weight. After i.v. administration, elimination half-life of enrofloxacin was 4.4 h and volume of distribution was 2.3 $L.kg^{-1}$ body weight. Enrofloxacin was rapidly metabolised to ciprofloxacin. The half-life of

ciprofloxacin paralleled that of the parent drug, its concentration in serum reached 20-35% of that of the parent drug. After i.m. administration, elimination half-life of enrofloxacin was longer (9.9 h) than after i.v. administration. Mean absorption time of enrofloxacin was also long (9.9 h). No statistically significant differences were found when half-life and mean residence time of antimicrobial activity were compared with those of enrofloxacin and ciprofloxacin from chemically analysed data (Kaartinen *et al.*, 1997).

In foal, Bermingham *et al.* (2000) noted mean ± SD total area under the curve ($AUC_{0-\infty}$) was 48.54 ± 10.46 $\mu g.h.ml^{-1}$, clearance was 103.72 ± 0.06 $ml.kg^{-1}.h^{-1}$, half life ($t_{1/2}\beta$) was 17.10 ± 0.09 h, and apparent volume of distribution was 2.49 ± 0.43 $L.kg^{-1}$ after i.v. dose of 5 $mg.kg^{-1}$. Compared with adult horses given 5 mg of enrofloxacin/kg i.v., foals have higher $AUC_{0-\infty}$, longer $t_{1/2}\beta$, and lower clearance. Concentration of ciprofloxacin was negligible. Using a target C_{max} to minimum inhibitory concentration ratio of 1 : 8 to 1 : 10, computer modeling suggests that 2.5 to 10 mg of enrofloxacin/kg administered every 24 h would be effective in foals, depending upon minimum inhibitory concentration of the pathogen.

In mare, Haines *et al.* (2000) conducted kinetic study of enrofloxacin after i.v. administration at a single dose rate of 7.5 $mg.kg^{-1}$ body weight. At 5 min after injection, mean serum concentration was 9.04 $\mu g.ml^{-1}$ and decreased to 0.09 $\mu g.ml^{-1}$ by 24 h. Elimination

half-life was 5.33 ± 1.05 h and the area under the serum concentration Vs time curve (AUC) was 21.03 ± 0.19 mg. h. L^{-1}. Bio-availability was calculated at 78.29 ± 16.55 %. The minimum inhibitory concentration of enrofloxacin required to inhibit 90% of isolates (MIC_{90}) was 0.25 $\mu g.ml^{-1}$ for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., and *Pasteurella* spp. were determined in equine bacterial culture specimens.

Sheep :

Pugliese *et al.* (1991) detected enrofloxacin in serum for up to 4 h after i.v. and 8 h after i.m. injection at the dose rate of 2.5 $mg.kg^{-1}$. With the i.m. route, the maximum serum concentration was reached in 1 h. With both routes, enrofloxacin was detected in milk in 1 h and persisted for 8 h.

In sheep, Mengozzi *et al.* (1996) noted a rapid distribution phase and a slower elimination phase with a half life ($t_{½}$ β) of 3.73 ± 0.44 h after i.v. dose of 2.5 $mg.kg^{-1}$. When the same dose was administered i.m. the drug was rapidly absorbed, reaching mean peak plasma concentration in 1.2 ± 0.11 h ; after that time, it appeared to decrease, with a half life of 3.65 ± 0.31 h. The bioavailability (F) of enrofloxacin by i.m. route was calculated to be 85.28 ± 3.40%. Volume distribution (Vd_{ss}) was noted to be 3.02 ± 0.22 and 3.03 ± 0.31 $L.kg^{-1}$ for i.v. and i.m. route. The total body clearance (Cl_B) values of 0.55 ± 0.14 and 0.62 ± 0.33 $L.kg^{-1}.h^{-1}$ for i.v. and i.m. administration, respectively.

Goat :

Sudha Kumari (1998) conducted kinetic study of enrofloxacin after single i.v. and s.c. administration of enrofloxacin in healthy lactating goat at the dose rate of 5 $mg.kg^{-1}$ body weight. They noted mean absorption half life ($t_{½}$ Ka) and distribution half life ($t_{½}$ α) of 0.60 ± 0.01 and 0.20 ± 0.03 h in goat. Elimination half life ($t_{½}$ β) values were also observed as 2.82 ± 0.33 and 1.42 ± 0.15 h for i.v. and s.c. administration, respectively. The rate constant of drug transfer from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were noted to be 0.436 ± 0.133, 0.639 ± 0.087 and 0.577 ± 0.137 h^{-1} respectively, for i.v. route. Vd_{area} of 2.34 ± 0.54 & 5.26 ± 1.23 $L.kg^{-1}$ and the total body clearance (Cl_B) 9.40 ± 1.36 & 43.3 ± 9.10 $ml.kg^{-1}.min^{-1}$ have been found for i.v. and s.c. administration, respectively.

Rao *et al.* (2001) noted that following single i.m. injection of enrofloxacin in goats at the dose rate of 2.5 $mg.kg^{-1}$, the plasma concentrations of enrofloxacin and ciprofloxacin were determined simultaneously by a HPLC method. The peak concentration (C_{max}) of enrofloxacin (1.13 $\mu g.ml^{-1}$) and ciprofloxacin (0.24 $\mu g.ml^{-1}$) were observed at 0.8 and 1.2 h, respectively. The elimination half life ($t_{1/2}$ β), Vd_{area}, Cl_B and MRT of enrofloxacin were 0.74 h, 1.42 $L.kg^{-1}$, 1329 $ml.h^{-1}.kg^{-1}$ and 1.54 h, respectively. The $t_{1/2}$ β, AUC and MRT of ciprofloxacin were 1.38 h, 0.74 $\mu g.ml^{-1}$ and 2.73 h, respectively. The metabolic conversion of enrofloxacin to ciprofloxacin was appreciable

(36%) and the sum of the plasma concentration of enrofloxacin and ciprofloxacin was maintained at or above 0.1 $\mu g.ml^{-1}$ for up to 4 h.

Pig :

Kuhn (1993) reported that following single i.v. injection of enrofloxacin in pig at the dose rate of 2.5 $mg.kg^{-1}$, the peak plasma concentration of 0.68 $\mu g.ml^{-1}$ was achieved at 225 minutes of injection. He also reported that since the amount in urine exceeded 4 $mg.L^{-1}$ during 12 h after injection, the drug may be suitable for treating urinary tract infection.

Anadon *et al.* (1999) noted that following single i.v. and i.m. injection of enrofloxacin in 8 pigs at the dose rate of 2.5 $mg.kg^{-1}$, mean ± S.D. elimination half life and mean residence time of enrofloxacin in plasma were 9.64 ± 1.49 and 12.77 ± 2.15 h, respectively, after i.v. administration and 12.06 ± 0.68 and 17.15 ± 1.04 h, respectively, after i.m. administration. Half-life at alpha phase of enrofloxacin was 0.23 ± 0.05 and 1.94 ± 0.70 h for i.v. and i.m. administration, respectively.

Dog :

Kung *et al.* (1993) noted mean $t_{½}$ β of 2.4 h, mean total body clearance of 27.1 $ml.min^{-1}.kg^{-1}$ and mean Vd_{ss} of 7 $L.kg^{-1}$ were obtained after i.v. administration of enrofloxacin at the dose rate of 5 $mg.kg^{-1}$.

Kanemaki *et al.* (1995) noted the half life of enrofloxacin to be 3 h at the dose rate of 5 $mg.kg^{-1}$ body weight and in vitro protein binding was 32.6%.

Camel :

Enrofloxacin was administered i.v., i.m. and s.c. to normal camels and to camels deprived of water for 14 days. Camels lost an average 12.5% of body weight at the end of the water deprivation period. The disposition kinetics of i.v. administered drug in normal and water-deprived camels were similar. After s.c. administration, the mean absorption half-life in the water-deprived camels was significantly longer than in the normal camels but systemic availability was significantly greater in normal camels (0.92 compared with 0.65 in water-deprived camels). In normal camels, urinary recovery at 12 h after i.v. and s.c. dosing was 25 and 15%, respectively, and the extent of serum protein binding ranged between 1.7% at 1.8 $\mu g.\ ml^{-1}$ and 24% at 0.33 $\mu g.ml^{-1}$. Serum drug concentrations were consistently higher than in the milk. The AUC milk/AUC serum ratios were 0.27 and 0.39 after i.v. and i.m. drug administration, respectively. An i.m. or s.c. treatment regimen of 2.5 $mg.kg^{-1}$ at 12 h is suggested for clinical and bacteriological efficacy trials with enrofloxacin in normal and dehydrated camels (Gavrielli *et al.*, 1995).

Rabbit :

Scheer *et al.* (1990) reported MIC of enrofloxacin against rabbit isolates of *E. coli, Bordetalla, Yersinia* and *Staphylococci* ranged from 0.3-0.6 $\mu g.ml^{-1}$. Oral administration at 5 $mg.kg^{-1}$ gave blood concentration of 0.5-0.6 $\mu g.ml^{-1}$ (0.3 - 0.6 $\mu g.ml^{-1}$ after administration in drinking water), while s.c. injection produced 1.3 $\mu g.ml^{-1}$ after 30-60 min. Tissue concentrations were higher than serum concentrations.

Broome *et al.* (1991) noted over all elimination half lives of i.v., s.c. and oral routes of administration of enrofloxacin (5 mg. kg^{-1}) in rabbit were 2.5, 1.71 and 2.41 h, respectively. The half life of absorption for oral dosing was 26 times the half life of absorption after s.c. dosing (7.73 h vs 0.3 h). The observed time to maximum serum concentration was 0.9 h after s.c. dosing (2.07 $\mu g.ml^{-1}$) and 2.3 h after oral administration (0.452 $\mu g.ml^{-1}$). Mean residence times were 1.55 h for i.v. injections, 1.46 h for s.c. dosing and 8.46 h for oral administration. Enrofloxacin was widely distributed in rabbit as suggested by the volume of distribution being 2.12 $L.kg^{-1}$. The volume of distribution at steady state was 0.93 $L.kg^{-1}$. Compared with i.v. administration, bioavailability was 77% after s.c. dosing and 61% for gastrointestinal absorption.

Cabanes *et al.*(1992) reported the pharmacokinetics and bioavailability of enrofloxacin determined after i.v. and i.m. administration of 5 $mg.kg^{-1}$ of body weight in rabbits. They used nonlinear least square regression methods and the data obtained were best described by a 2-compartment open model. They noted that after i.v. administration, a rapid distribution phase was followed by a slower elimination phase, with a half life of 131.5 ± 17.6 min. The mean body clearance rate was 22.8 ± 6.8 ml. $min^{-1}.kg^{-1}$ and the mean volume of distribution (Vd_{area}) was 3.4 ± 0.9 $L.kg^{-1}$. It was thought that this large volume of distribution and the K_{12}/K_{21} ratio close to 1 indicated that enrofloxacin was widely distributed in the body but not retained in tissues. The mean extent of i.m. absorption was 92 ± 11% and maximum plasma concentration of 3.04 ± 0.34 μg. ml^{-1} was detected approximately 10 min after administration.

Chicken :

In chicken, Anadon *et al.* (1995) noted shorter distribution half life of 0.070 ± 0.001 h and a longer elimination half life of 10.29 ± 0.45 h after i.v. administration (10 $mg.kg^{-1}$). In similar dose after oral administration, a comparatively longer absorption half life of 14.23 ± 0.46 h and the bioavailability of 64.0 ± 0.2% were noted. Volume distribution (Vd_{area}) of 4.31 ± 0.15 and 5.94 ± 0.20 $L.kg^{-1}$ and total body clearance (Cl_B) of 0.29 ± 0.02 and 0.288 ± 0.02

$L.h^{-1}.kg^{-1}$ were obtained after i.v. and oral administration of enrofloxacin, respectively. The values of rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}), and elimination from central (Kel) compartment were noted to be 6.13 ± 0.21, 0.19 ± 0.01 and 3.46 ± 0.09 h^{-1} after i.v. administration of enrofloxacin.

The pharmacokinetics of enrofloxacin and ciprofloxacin was investigated in broiler chickens by Garcia Ovando *et al.* (1999). Each antimicrobial was administered intravenously at a dose of 5 mg. kg^{-1} body weight. The concentration of enrofloxacin and ciprofloxacin in plasma were determined by HPLC. Plasma concentrations versus time were analysed by a compartmental independent pharmacokinetic model that provided the most important kinetic parameters. Statistically significant differences between the two antimicrobials were found for most of the pharmacokinetic parameters : Area under the curve (AUC), area under first moment curve (AUMC), mean residence time (MRT), total body clearance (Cl_B), volume of distribution beta (Vd beta) and volume of distribution at the steady state (Vd_{ss}). Both antimicrobials were widely distributed in chickens throughout the body with a mean Vd_{ss} of 1.98 ± 0.18 $L.kg^{-1}$ for enrofloxacin and 4.04 ± 0.69 $L.kg^{-1}$ for ciprofloxacin. The Cl_B for ciprofloxacin was five times higher than that obtained for enrofloxacin. AUC, MRT and the diminished half times for

enrofloxacin were two to four times higher than those obtained for ciprofloxacin. These results indicate that ciprofloxacin remains in the body for less time than the other quinolone. This characteristic of ciprofloxacin suggests the advantage of a shorter withdrawal time for food producing animals treated with this antimicrobial.

The plasma pharmacokinetics of danofloxacin and enrofloxacin in broiler chickens was investigated following i.v. administration at dose rate of 5 $mg.kg^{-1}$ body weight for danofloxacin and 10 $mg.kg^{-1}$ body weight for enrofloxacin (Knoll *et al.*, 1999). Pharmacokinetic parameter values calculated by non-compartmental methods were similar for danofloxacin compared to enrofloxacin with respect to elimination half-life ($t_{½}\beta$; approximately 6-7 h), mean residence time (MRT; 6-9 h). However, values were two fold higher for body clearance (Cl_B ; 24 versus 10 ml. $min^{-1}.kg^{-1}$) and volume of distribution at steady state (Vd_{SS} ; 10 versus 4 $L.kg^{-1}$) was noted.

The disposition kinetics of enrofloxacin following single i.v. administration in healthy and *E. coli* infected broilers at the dose rate of 10 $mg.kg^{-1}$ body weight. The elimination half life ($t_{½}\beta$) was 4.75 vs 3.63 h ; mean residence time (MRT) was 6.72 vs 4.90 h ; apparent volume of the central compartment (Vc) was 1.11 vs 1.57 $L.kg^{-1}$, rate constant for transfer from peripheral to central compartment (K_{21}) was 1.15 vs 1.41 h^{-1} and total body clearance (Cl_B) was 0.35 vs 0.53 $L.h^{-1}.kg^{-1}$ in healthy and infected birds, respectively (Soliman, 2000).

IMPORTANT KINETIC PARAMETERS OF ENROFLOXACIN IN DIFFERENT SPECIES

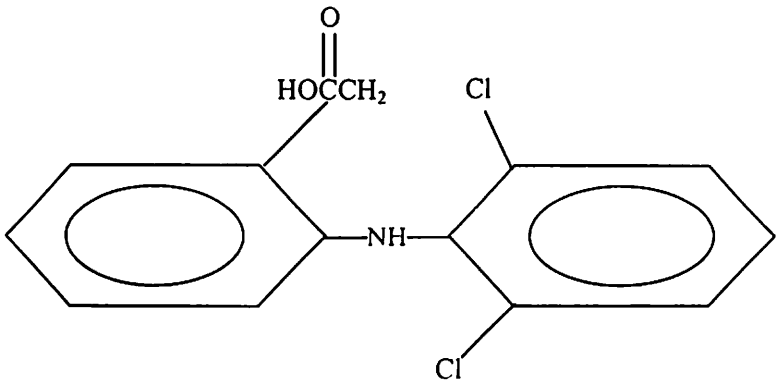
Species	Absorption half life ($t_{1/2}$ Ka) (h)	Distribution half life ($t_{1/2}$ α) (h)	Elimination half life ($t_{1/2}$ β) (h)	Volume distribution (L.kg.$^{-1}$)	Total body clearance (ml.kg^{-1}.min^{-1})	Dose (mg.kg^{-1})	Route of administration	Reference
Bovine								
1. Cow	-	-	0.734 (enro) 0.934 (cipro)	0.6	-	5	i.v.	Gardorfer (1991)
	-	-	0.734	0.6	-	5	i.v.	Kaartinen *et al.* (1994)
	-	-	1.7	1	-	5	i.v.	Kaartinen *et al.* (1995)
	-	-	5.9	-	-	5	i.m.	
	-	-	5.6	-	-	5	s.c.	
2. Calf								
(a) New born	-	-	6.6	1.8	3.17	2.5	i.v.	Kaartinen *et al.* (1997)
(b) One week old	-	-	4.9	2.3	6.5	2.5	i.v.	
3. Buffalo bull	0.262±0.099	-	1.97±0.23	0.61±0.13	Approx. 3.5	5	i.m.	Verma *et al.* (1999)
Equine								
1. Horse	-	-	6.5	2	-	5	i.v.	Zehe (1990)
	-	0.68	5.94	1.22±0.07	Approx. 2.34	2.5	i.v.	Giguere *et al.* (1996)
	-	0.63	6.09	0.77±0.11	Approx. 1..5	5	i.v.	
	-	-	4.4	2.3	-	5	i.v.	Kaartinen *et al.* (1997)
2. Foal	-	-	17.10±0.09	2.49±0.43	Approx 1.73	5	i.v.	Bermingham *et al.* (2000)
3. Mare	-	-	5.33±1.05	-	-	7.5	i.v.	Haines *et al.* (2000)
Sheep	-	-	3.73±0.44	3.02±0.22	9.17±2.4	2.5	i.v.	Mengozzi *et al.* (1996)
	-	-	3.65±0.31	3.03±0.31	10.34±5.5	2.5	i.m.	
Goat	0.60±0.01	-	1.42±0.15	5.26±1.23	43.3±9.10	5	s.c.	Sudha Kumari (1998)
	-	0.20±0.03	2.82±0.33	2.34±0.54	9.40±1.36	5	i.v.	
	-	-	0.74 (enro) 1.38 (cipro)	1.42	22.11	2.5	i.m.	Rao *et al.* (2001)
Pig	-	0.23±0.05	9.64±1.49	-	-	2.5	i.v.	Anadon *et al.* (1999)
	1.94±0.70	-	12.06±0.68	-	-	2.5	i.m.	
Dog	-	-	2.4	7	27.1	5	i.v.	Kung *et al.* (1993)
Rabbit	-	-	2.5	2.12	-	5	i.v.	Broome *et al.* (1991)
	0.3	-	1.71	-	-	5	s.c.	
	7.73	-	2.41	-	-	5	oral	
	-	-	2.19	3.4±0.9	22.8±6.8	5	i.v.	Cabanes *et al.* (1992)
Chicken	-	0.07±0.001	10.29±0.45	4.31±0.15	Approx. 4.83	10	i.v.	Anadon *et al.* (1995)
	1.43±0.1	-	14.23±0.46	5.94±0.20	Approx 4.8	10	oral	
	-	-	-	1.98±0.18 (enro) 4.04±0.69 (cipro)	-	5	i.v.	G.Ovando *et al.* (1999)
	-	-	7	4	10	10	i.v.	Knoll *et al.* (1999)
	-	-	4.75	1.11	5.83	10	i.v.	Soliman (2000)

DICLOFENAC

Diclofenac is a potent nonsteroidal anti inflammatory drug (NSAID), which is widely used in human and veterinary practice. It is the first of a series of phenyl acetic acid derivatives that has been developed as an anti inflammatory agent. It is also an analgesic compound with good antipyretic and uricosuric properties (Maier *et al.*, 1979).

1. Chemistry :

Chemically, diclofenac is a phenyl acetic acid derivative. The chemical structure is as follows :



Empirical formula = $C_{14}H_{23}O_2Cl_2N$

Molecular weight = 307

2. Therapeutic Uses :

Diclofenac is used in veterinary practice for treating non-descriptive pyrexia, painful conditions due to acute and chronic inflammation, muscular pain, joint pain, neuralgia, soft tissue injuries, such as sprain or strain and immobility associated with lameness, arthritis, myositis etc.

3. *Mode of Action :*

Diclofenac posses analgesic, antipyretic and anti-inflammatory properties. It inhibits the cyclo-oxygenase pathway in the metabolism of arachidonic acid and thus exerts its anti-inflammatory action by blocking the synthesis of prostaglandins, prostacycline and thromboxane products. Diclofenac also inhibits the lipo-oxygenase pathway and thereby reducing the production of leukotrienes and monohydroxy acids, which are associated with the inflammatory processes. It also reduces polymorph chemotaxis and production of lysosomal enzymes and super-oxide radicals, thereby reducing tissue destruction in inflammatory reactions. It also inhibits bradykinin, an important mediator of pain and inflammation. Diclofenac suppresses hyperthermia through its action on the thermo-regulatory centre in hypothalamus. In rats with yeast induced fever, diclofenac reduced body temperature by 1.5 ^{0}C in lower doses than did indomethacin, ibuprofen, phenylbutazone, naproxen and aspirin.

4. *Pharmacokinetics and Metabolism :*

Diclofenac is rapidly and completely absorbed after oral administration and peak concentrations in plasma are reached within 2 to 3 hours. Administration with food slows the rate but does not alter the extent of absorption. The drug gets completely absorbed following i.m. injection. C_{max} & AUC are dose related in the range of 25-150 mg. It is extensively bound to plasma proteins (99%) and its

half life in plasma is 1 to 2 hours. Diclofenac accumulates in synovial fluid after oral administration, that may be the possible reason behind the longer duration of therapeutic effect than the plasma half-life. Diclofenac is metabolized in the liver to 4-hydroxy diclofenac, the principal metabolite and other hydroxylated forms. The metabolites are excreted in the urine (65%) and bile (35%). Apart from liver, bile and kidney, high levels of diclofenac are found in blood, heart and lungs.

5. *Kinetic Studies :*

Pharmacokinetic studies on diclofenac were conducted in different species. They are noted as follows :

Man :

In man, Willis *et al.* (1979) noted the lag time between dosing and appearance of drug in plasma varied between 1.0 and 4.5 h after oral doses. Peak plasma levels ranged from 1.4 to 3.0 $\mu g.ml^{-1}$. The mean terminal drug half-life in plasma was 1.8 h after oral dose and 1.1 h after i.v. dose. He noted availability (oral) 54 ± 2%, urinary excretion less than 1%, bound in plasma more than 99.5%, clearance 4.2 ± 0.9 $ml.min^{-1}.kg^{-1}$ and volume distribution 0.17 ± 0.11 $L.kg^{-1}$. After i.v. injection, plasma levels of diclofenac fell rapidly and were below the limits of detection at 5.5 h post dosing.

Kurowski (1988) noted oral bioavailability of 72.9% with an average lag time of 2.2 h. Peak plasma concentrations amounted to 2.9 $\mu g.ml^{-1}$ after 3.1 h as compared to 2.15 $\mu g.ml^{-1}$ after 20-30 min. following an intramuscular injection of 75 mg. Diclofenac was excreted with an average half-life of 1.15 h. The bioavailability of the three i.m. injectable solutions, as calculated from the area under the curve (AUC), did not differ significantly.

Pig :

The pharmacokinetics and metabolism of diclofenac was studied in yucatan minipigs after i.v. administration of 25 and 50 mg and after oral administration of 50 mg in a solution of 50 ml buffer, 50 ml water & 200 ml water and the results were compared to historical data in man. The absolute bioavailability after oral administration of 50 ml buffer, 50 ml water, and 200 ml water solutions were 107, 97, and 107% respectively as compared to approximately 50% in man. The total plasma clearance in minipigs was five fold slower than in man (57 ± 17 vs 252 ± 54 $ml.h^{-1}.kg^{-1}$). The volume of distribution of the central compartment (Vdc) was 40% less in man than in pigs (39 vs 67 $ml.kg^{-1}$). The terminal half-lives of the parent drug were similar in pigs (2.4 h) and man (1.8 h). The rate of oral drug absorption increased in the order of 50 ml aqueous, 200 ml aqueous and 50 ml buffered solutions (Ka = 0.52 ± 0.11, 0.59 ± 0.13 and 1.2 ± 0.7 h^{-1}, respectively) as observed by Oberle *et al.* (1994).

Rat :

In rat, biliary excretion of the drug (unchanged and conjugated) was detected in bile duct cannulated rats were 27.2 and 31.2% and only 4.7 and 5.4 % excreted in the bile after i.v. and intraduodenal administration, respectively. Maximum plasma concentration was reached within 2 min after intraduodenal dosing. Bioavailability in the bile duct-cannulated rats was 71% after indraduodenal dose where as in normal animal was 79% after oral dose and 106% after intraduodenal dose (Peris - Ribera *et al.*, 1991).

GENERAL PHARMACOKINETICS

Pharmacokinetics often referred to as disposition kinetics, helps in knowing absorption, distribution, metabolism and excretion of drugs (Dost, 1953). According to Wagner (1968), the aim of pharmacokinetics is to study the time concentration course of drugs and their metabolites in various body fluids, tissues & excretion and interpretation of such data based on suitable pharmacokinetic models (Compartment models).

The compartment model is a hypothetical structure which can be used to characterise with reproducibility of behaviour and fate of drugs in a biological system, when administered by certain route in a particular dosage form. In pharmacokinetic studies, compartment is an entity which has a definite volume and in that concentration, a drug exists at any time. The disposition kinetics of

drug is described either by one compartment or multi-compartment open models. Body distributes the drugs in all tissues widely at varying rates and is therefore, designated as open system. An open compartment model shows free movement of a drug from one compartment to another (i.e. blood to tissue and vice-versa).

One compartment open model :

When the distribution of drug from central to peripheral compartment is very rapid, the drug is said to follow one-compartment open model. Any change in drug concentration in the blood reflects directly the quantitative change in its tissue level. Baggot (1974) reported that the rate of drug elimination from the body is proportional to the concentration of the drug in blood.

In one compartment open model, if the plasma concentration-time profile is plotted from the peak concentration onwards on a semilogarithmic scale, a straight line is obtained (Sams, 1978) and the plasma drug level declines according to following equation :

$$C_P = Be^{-\beta t} \qquad \text{.....Eq.1}$$

where,

C_P = Concentration of drug in plasma.

B = Extrapolated zero time intercept of mono exponential curve.

β = Over all elimination rate constant.

t = Time elapsed after drug administration.

e = Base of natural logarithm.

Baggot (1977) reported that the one compartment open model is particularly useful in describing the time course of most drugs in plasma following extravascular (oral/i.m./s.c.) administration.

Two compartment open model :

The pharmacokinetics of most of the drugs following i.v. administration are accurately described by two compartment open model. Baggot (1974) stated that in two compartment open model the drug distribution is instataneous and homogeneous into the central compartment (such as blood and other readily accessible tissues like liver and kidney) and more slowly into the peripheral compartment (comprising of less perfused organs and tissues such as muscles and fat). This indicates that distribution and elimination processes follow the first order kinetics and elimination takes place exclusively from central compartment. In two compartment open model, semi-logarithmic plot of plasma drug concentration against time shows a biphasic curve. The initial steep decline in plasma drug concentration is mainly due to the distribution of drug from central to peripheral compartment. Once apparent distribution is established, the gradual decline is obtained mainly by irreversible elimination of drug from the central compartment.

The drug concentration in plasma is expressed by the following biexponential mathematical expression as a function of time :

$$C_P = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{.....Eq.2}$$

where,

C_P= Plasma concentration of the drug.

A = Zero time intercept of distribution phase.

B = Zero time intercept of elimination phase.

α = Distribution rate constant.

β = Elimination rate constant.

e = Base of natural logarithm.

t = Time elapsed after drug administration.

The values of A, B, α and β are essential in calculating other kinetic rate constants (K_{12}, K_{21} and Kel) in two compartment open model. The values of these rate constants give an idea of relative contribution of distribution and elimination processes to the drug concentration-time data (Baggot,1977).

Three or Multi compartment open model :

The disposition kinetics of some drugs may also follow three or multiple compartment model. In three compartment open model, the semilogarithmic plot of plasma drug concentrations against time shows a triphasic curve. The initial sharp decline in plasma concentration against time is due to distribution of drug form

blood to highly perfused tissue compartment (peripheral I). The gradual decline is because of distribution of drug from central to moderately blood supplied organs (peripheral II). The drug concentration in plasma following single intravenous administration is expressed by the following triexponential mathematical formula as a function of time :

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad \text{.....Eq.3}$$

The additional constants C and γ are calculated by using residual methods. These constants may be employed to estimate K_{13} and K_{31} (Gibaldi and Perrier, 1975).

Non-compartmental model :

Non-compartmental method does not require the assumption of a specific compartmental division of body to study the disposition pattern of drug and/or its metabolite. This method is applied for linear/non-linear pharmacokinetics. In compartmental open model, some of the parameters, such as estimation of bioavailability, total body clearance, apparent volume of distribution are derived from methods based on non-compartmental analysis. Only the fact is that this term is comparatively new although being used for long time. In case of compartmental open model, the parameters are derived from the best-fitting curve where as, in non-compartmental model the methods are statistical involving derivation and integration. The whole analysis is based on statistical moment theory (Srivastava and Deore, 1999).

Pharmacokinetics of clinical importance :

Clinically, the pharmacokinetic studies consist of :

(a) Calculation of various kinetic parameters following different routes of administration.

(b) Estimation of drug dosage regimen in a particular species of animal.

(c) Determination of drug withdrawal period for drug residues in milk and tissues of food producing animals.

Some important pharmacokinetic parameters :

1. *Absorption rate constant (Ka) and absorption half-life ($t_{½}$ Ka) :*

These denote the rate of absorption (faster or slower) of a drug from its site after extravascular (i.m./s.c./oral) administration.

2. *Distribution rate constant (α) and distribution half-life ($t_{½}$ α) :*

These parameters indicate the rate of distribution (faster or slower) of a drug from plasma to body fluids and tissues following i.v. administration.

3. *Elimination rate constant (β) :*

Baggot (1977) and Mercer *et al.* (1977) stated that the overall elimination rate constant (β) is the most essential kinetic parameter since it is employed to determine :

(i) the elimination half-life ($t_{½}$ β)

(ii) the volume of distribution by area method (Vd_{area})

(iii) the total body clearance (Cl_B)

(iv) the drug withdrawal period for drug residues in milk and tissues of food producing animals.

4. Elimination half-life ($t_{1/2}$ β) :

Gibaldi and Weintraub (1971) defined that the elimination half-life is the time required to reduce the drug concentration in plasma or serum to its half during the elimination phase of the drug concentration time profile. This means that doubling the dose does not double the duration of action of drug but increases it by one half life. It is inversely proportional to the overall elimination rate constant. It is used to calculate the duration of drug action in the body. The half life of a first order process is independent of the dose of drug as well as the route of administration. Knowledge of the half-life of a drug is extremely helpful in designing the rational dosage regimen.

5. Volume of distribution :

The apparent volume of distribution is an important pharmacokinetic parameter used in the kinetic characterization of a drug. It is a hypothetical volume of body fluid that would be required to dissolve the total amount of the drug to attain the same concentration as that found in the blood. Riegelman *et al.* (1968) stated that the calculated value of volume of distribution is not dependent upon the method used for its calculation if the drug distributes truly according to one compartment open model. The apparent volume of distribution indicates the amount of distribution of a drug without providing any clue, whether the drug is uniformly distributed or restricted to certain tissues (Baggot, 1977). A large

volume of distribution (>1 $L.kg^{-1}$) indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. A small volume of distribution indicates that the drug is restricted to certain fluid compartments, like plasma water, extracellular fluid etc. This is due to the high protein binding or low lipid solubility of a drug.

6. Total body clearance (Cl_B) :

Another important pharmacokinetic parameter is the total body clearance (Cl_B) which is the sum of the clearance of each eliminating organ, particularly liver and kidney. The half life of a drug is a complex function which depends upon the process of drug distribution, bio transformation and excretion. The parameter, body clearance, on the other hand is independent of these processes and indicates the rate of drug removal from the body. Unlike β and $t_{½}$ β that are hybrid constants and depend upon K_{12}, K_{21} and Kel, the total body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

It is reported that the various constants, namely A, α, B, β, $t_{½}$ α, $t_{½}$ β and Vd_{area} etc. change disproportionally with the magnitude of the elimination rate constant from central compartment (Kel) and hence, should not be employed individually as a direct or sole measure of a change in drug elimination or distribution (Jusko and Gibaldi, 1972).

Dosage Regimen :

Dose is a quantitative term estimating the amount of drug which must be administered to produce a particular biological response i.e. to attain optimum effective concentration of drug in the body fluids. Maintenance of therapeutic concentration of a drug in the body requires the administration of maintenance dose at a particular dose interval after administering the priming or loading dose, so that plasma drug concentration must be above a minimum effective level and below a level producing excessive side effects and toxicity. Thus, the objective of a multiple dosage regimen is to maintain the plasma concentration of the drug within the limits of the maximum safe concentration and the minimum effective levels.

KINETIC INTERACTION OF ANTIMICROBIALS WITH NON-STEROIDAL ANTI-INFLAMMATORY, ANALGESICS AND ANTIPYRETICS AGENTS :

Antimicrobials and non-steroidal anti-inflammatory, analgesics and antipyretics agents are frequently used concomitantly and pharmacokinetic interactions between them have been described (Joly *et al.*, 1988 ; Mueller *et al.*, 1993 ; Manna *et al.*, 1994 ; Nergelius *et al.*, 1997 ; Sudha Kumari, 1998 ; Tang *et al.*, 1999 and Varma *et al.*, 2000).

The effect of diclofenac on the pharmacokinetics of the three cephalosporins viz., ceftriaxone, cefotiam and cefmenoxime was

studied in rabbits by Joly *et al.* (1988). Ceftriaxone concentrations at 1, 2, 4, 6, 12 and 24 h and AUC in serum increased significantly ($P < 0.05$) when this antimicrobial was administered in conjunction with diclofenac. Diclofenac increased significantly ($P < 0.05$) the serum terminal half life ($t_{½}$ β) of ceftriaxone and non-significantly that of cefotiam but not cefmenoxime.

The mean pharmacokinetic characteristics of cyclosporine were unchanged during coadministration with diclofenac was studied in man by Mueller *et al.* (1993). A single oral dose of 300 mg cyclosporine was administered alone and on day 8 of multiple oral dosing of 50 mg diclofenac every 8 h. Serial blood samples were obtained over 48 h after each cyclosporine dose and over a dosing interval for diclofenac on day 7 (diclofenac alone) and day 8 (co-administration of diclofenac with cyclosporine). Based on area under the curve (AUC) comparison, lack of a pharmacokinetic interaction was conclusively demonstrated for the extent of cyclosporine absorption. The diclofenac maximum plasma concentration and AUC over a dosing interval were significantly increased during co-administration ; however, a straight forward interpretation of the statistical result was confounded by pronounced variability in diclofenac pharmacokinetics. The results underscore the need for continued caution when cyclosporine and diclofenac are co-administered.

Modification of the disposition kinetics of paracetamol by oxytetracycline in goats was carried out by Manna *et al.* (1994). They observed that the C_{max} value of paracetamol alone (128.0 ± 8.0 $\mu g.ml^{-1}$) was significantly ($P < 0.01$) higher as compared to the combined therapy with oxytetracycline (46.8 ± 3.4 $\mu g.ml^{-1}$) at 0.03 h post i.v. drug administration. Paracetamol persisted in the blood till 2 h and 4 h for alone and combined therapy respectively. The C_p^0 value of paracetamol alone (163.3 ± 9.9 $\mu g.ml^{-1}$) was significantly ($P < 0.01$) higher compared to combined therapy (56.0 ± 2.6 $\mu g.ml^{-1}$). The α and $t_{½}$ α values of paracetamol alone were higher and lower, respectively, as compared to combined administration. On the other hand $t_{½}$ β, Vd, Vd_B, Vd_{area} and Vd_{ss} values of combined therapy was significantly higher ($P < 0.02$) from the corresponding values of paracetamol alone.

No effect of diclofenac on the pharmacokinetics of cloxacillin was shown in man by Nergelius *et al.* (1997). Total plasma clearance of cloxacillin was with placebo 219 ± 51 (mean ± S.D.) and with diclofenac 212 ± 39 ml/min/1.73 m^2 (ns) ; renal clearance was 97 ± 21 and 96 ± 24 ml/min/1.73 m^2, respectively (ns). The terminal $t_{½}$ of cloxacillin was 1.03 ± 0.42 h with placebo, and 1.12 ± 0.37 h with diclofenac (ns). Thus, diclofenac does not alter cloxacillin pharmacokinetics.

Pharmacokinetics of enrofloxacin (@ 5 $mg.kg^{-1}$) when given alone and in combination with paracetamol (@ 50 $mg.kg^{-1}$) by i.v. route in six goats was carried out by Sudha Kumari (1998). She observed that the mean therapeutic concentration (0.12 $\mu g.ml^{-1}$) in plasma was maintained up to 10 h for enrofloxacin and 6 h for enrofloxacin with paracetamol. Significantly higher values were obtained for zero time concentration in distribution phase (A) and theoretical zero time concentration (C_p^0) were 19.60 ± 3.92 and 21.52 ± 4.12 $\mu g.ml^{-1}$, respectively in combined administration as compared to single administration (3.37 ± 0.79 and 5.27 ± 0.96 $\mu g.ml^{-1}$, respectively). Significantly higher elimination rate constant (β) and lower elimination half life ($t_{½}$ β) of 0.456 ± 0.067 h^{-1} and 1.70 ± 0.26 h, respectively in combination as compared to single administration (0.270 ± 0.041 h^{-1} and 2.82 ± 0.33 h, respectively). The distribution half life (0.57 ± 0.17 h), AUC (18.90 ± 5.87 $mg.L^{-1}.h$), K_{12} (0.251 ± 0.079 h^{-1}), F_C (0.42 ± 0.09), T ≈ P (1.96 ± 0.48), Vd_{area} (1.10 ± 0.47 $L.kg^{-1}$) and Cl_B (9.22 ± 4.73 $ml.kg^{-1}.min^{-1}$) did not show any significant difference when enrofloxacin was given along with paracetamol as compared to enrofloxacin alone (0.60 ± 0.10 h, 9.85 ± 1.38 $mg.L^{-1}.h$, 0.436 ± 0.133 h^{-1}, 0.51 ± 0.06, 1.11 ± 0.22, 2.34 ± 0.54 $L.kg^{-1}$ and 9.40 ± 1.36 $ml.kg^{-1}.min^{-1}$, respectively).

The stimulation of diclofenac metabolism by interaction with quinidine was studied in monkeys by Tang *et al.* (1999). After a dose of diclofenac via portal vein infusion at 0.055 $mg.kg^{-1}.h^{-1}$, steady-

state systemic plasma drug concentrations in three male rhesus monkeys were 87, 104, and 32 $ng.ml^{-1}$, respectively (control). When diclofenac was coadministered with quinidine (0.25 $mg.kg^{-1}.h^{-1}$) via the same route, the corresponding plasma diclofenac concentrations were 50, 59 and 18 $ng.ml^{-1}$, representing 57, 56 and 56% of control values, respectively. In contrast, steady-state systemic diclofenac concentrations in the same three monkeys were elevated to 1.4 to 2.5 times when the monkeys were pretreated with L - 754, 394 (10 $mg.kg^{-1}$ i.v.), an inhibitor of cytochrome P - 450 (CYP) 3A. Further investigation indicated that the plasma protein binding (> 99%) and blood/plasma ratio (0.7) of diclofenac remained unchanged in the presence of quinidine. Therefore, the decreases in plasma concentrations of diclofenac after a combined dose of diclofenac and quinidine are taken to reflect increased hepatic clearance of the drug, presumably resulting from the stimulation of CYP 3A-catalyzed oxidative metabolism. Consistent with this proposed mechanism, a 2-fold increase in the formation of 5-hydroxy diclofenac derivatives was observed in monkey hepatocyte suspensions containing diclofenac and quinidine. Stimulation of diclofenac metabolism by quinidine was diminished when monkey liver microsomes were pretreated with antibodies against CYP 3A. Subsequent kinetic studies indicated that the K(m) value for the CYP - mediated conversion of diclofenac to its 5-hydroxy derivatives was little changed (75 vs 59 micro M), where as V (max) increased 2.5 fold in the presence of quinidine. These data

suggest that the catalytic capacity of monkey hepatic CYP 3A toward diclofenac metabolism is enhanced by quinidine.

Pharmacokinetics of enrofloxacin was studied in five cattle following i.m. administration (@ 5 $mg.kg^{-1}$) alone and along with diclofenac sodium (@ 0.8-1.0 $mg.kg^{-1}$). Therapeutic concentration (0.1 $\mu g.ml^{-1}$) in plasma was maintained up to 12 and 24 h for enrofloxacin and enrofloxacin along with diclofenac sodium, respectively. The plasma elimination half life (9.2 h), Vd_{area} (17.3 L. kg^{-1}), T_{max} (2 h), MRT (13.2 h) and body clearance (1.4 $L.kg^{-1}.h^{-1}$) was comparatively significantly higher when enrofloxacin was given along with diclofenac sodium as compared to enrofloxacin alone (5.9 h, 7.1 $L.kg^{-1}$, 0.4 h, 6.8 h and 0.82 $L.kg^{-1}.h^{-1}$, respectively). The AUC (3.8 $mg.h.ml^{-1}$) and C_{max} (0.2 $\mu g.ml^{-1}$) was significantly lower when enrofloxacin was administered along with diclofenac sodium compared to enrofloxacin given alone (5 $mg.h.ml^{-1}$ and 0.82 $\mu g.\ ml^{-1}$, respectively). Diclofenac sodium significantly ($P < 0.1$) reduced the plasma concentration of ciprofloxacin (as metabolite of enrofloxacin). Based on the pharmacokinetic parameters calculated, an intramuscular dosage regimen of enrofloxacin (Priming dose of 1.8 $mg.kg^{-1}$ followed by maintenance dose of 1.10 $mg.kg^{-1}$ every 8 h) to maintain a therapeutic concentration of 0.1 $\mu g.ml^{-1}$ is recommended in cattle (Varma *et al.*, 2000).

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Chapter - III

Materials and Methods

MATERIALS AND METHODS

In the present study, five clinically healthy female buffalo calves of non-descript breed between 12 to 18 months of age and 102 to 175 kg body weight were used. The buffalo calves were housed in the animal shed with concrete floor. The buffalo calves were maintained on dry fodder, cattle feed and greens. Water was given *ad lib*.

Experimental Design :

Enrofloxacin and diclofenac were administered separately in each of five healthy buffalo calves by intravenous (i.v.) route. An interval of 10-15 days was allowed to elapse before administration of next dose of the drug. After conducting kinetic study of these drugs alone, the drugs were administered together in combination by i.v. route to investigate the interaction of these drugs in buffalo calves.

Drugs Used :

Enrofloxacin and diclofenac were used in the present experiment. Enrocin® (10%), an injectable commercial preparation containing enrofloxacin in concentration of 100 $mg.ml^{-1}$ marketed by Ranbaxy Laboratories limited, India was used. Diclofenac, an injectable commercial preparation marketed under the trade name of Zobid® by Ambalal Sarabhai Enterprises Limited, India was used. Each ml of Zobid contains 25 mg of diclofenac sodium.

Collection of Biological Fluids and their Timings :

The samples of various biological fluids were collected after i.v. administration of drugs in healthy buffalo calves. The samples of plasma and urine were collected at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h but samples of urine were collected upto 48 h (at 30, 36 and 48 h).

(A) Blood :

Before collection of blood, the site around the jugular vein on either side of the neck of the animals were aseptically prepared. The site was sterilized prior to each collection with rectified spirit. Blood samples were collected in sterilized centrifuge tubes containing appropriate amount of sodium oxalate by venè-puncture with disposable 18G needles, at various above noted time intervals after drug administration. The blood samples were centrifuged at 3000 rpm for 10 min. for the separation of plasma. The plasma samples were then kept in a refrigerator until assay was carried out. For the preparation of standards, normal plasma prior to drug administration was also collected.

(B) Urine :

The urine samples were collected for analysis by introducing a sterile Foley's balloon catheter (No. 12) lubricated with

glycerine through urethra into the urinary bladder of the experimental buffalo calves with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 25-30 ml of sterile water through a syringe to keep the catheter in position. The opening of the catheter was blocked with a pressure clip to check dripping of urine. Prior to drug administration, urine sample was collected in a sterile test tube for the preparation of standards. After administration of the drug, the urine samples were collected in sterile test tubes at various above noted time intervals. The samples were kept in a refrigerator and were analysed in successive days.

Administration of Drugs :

Enrocin® 10% injection, containing 100 mg of enrofloxacin per ml was injected at the dose rate of 4 $mg.kg^{-1}$ body weight by i.v. route in each healthy buffalo calf. Zobid® injection containing 25 mg of diclofenac sodium per ml was administered at the dose rate of 1 $mg.kg^{-1}$ body weight by i.v. route in each healthy buffalo calf. After conducting kinetic study of enrofloxacin and diclofenac by i.v. route separately, both the drugs were administered together at the above stated dose rate in each animal by i.v. route to know the interaction of the drugs.

Estimation of Enrofloxacin and its active metabolite Ciprofloxacin :

1. **By High Performance Liquid Chromatography (HPLC) Method** :

Estimation of enrofloxacin and ciprofloxacin were done simultaneously by HPLC method described by Nielsen and Gyrd-Hansen (1997) and Kung *et al.* (1993) with slight modification as described below.

Apparatus :

The HPLC equipment used comprised of a HPLC pump (Model 515 – Waters), a dual wavelength absorbance detector (Model 2487 - Waters), a rheodyne manual injector with a 200 µl loop size and a data module (Model 746 – Waters). Chromatographic separations were performed using column 3.9 × 300 mm (µ Bondapak^{TM} C_{18} - Waters).

Chromatographic Conditions :

The flow rate was 0.6 ml.min^{-1}, the effluent wavelength was monitored at 278 nm, loop size was 200 µl, injection volume was 400 µl, the chart speed was 0.25 mm. min^{-1} and the detector sensitivity was 2.000 A.U.F.S (Absorbance under full scale) were adopted for HPLC analysis for enrofloxacin and its active metabolite ciprofloxacin.

Reagents :

All solvents used were of HPLC grade. All other chemicals and reagents were of analytical grade and freshly prepared triple distilled water was used. Enrofloxacin was obtained as gift from Ranbaxy Laboratories Limited, India which is manufactured under trade name of Enrocin® and ciprofloxacin was used from cadila Health care limited, India which is manufactured under trade name of Ciprobid®.

Mobile Phase :

The mobile phase comprised of acetonitrile : methanol : water (17 : 3 : 80 v/v/v) and water containing 0.4% phosphoric acid (85% v/v) and 0.4% triethylamine (v/v). The pH of mobile phase was 3 (approx).

Preparation of Standards of Enrofloxacin :

(a) In water :

Enrocin, an injectable commercial preparation containing enrofloxacin in concentration of 100 $mg.ml^{-1}$ was diluted in sterile triple distilled water to make different strengths viz., 40, 20, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 $\mu g.ml^{-1}$.

(b) In plasma :

From each standard solution of enrofloxacin in water, 0.1ml was added to a sterile vial containing 0.9 ml of plasma collected

prior to drug administration. This yielded enrofloxacin standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 $\mu g.ml^{-1}$ in plasma. Blank plasma containing no drug was also prepared.

Preparation of Standards of Ciprofloxacin :

(a) In water :

Ciprobid, an injectable commercial infusion preparation containing ciprofloxacin in concentration of 200 mg/100 ml i.e. 2 $mg.ml^{-1}$. Ciprofloxacin was diluted in triple distilled water to have different strengths viz. 40, 20, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 $\mu g.ml^{-1}$.

(b) In plasma :

From each standard solution of ciprofloxacin in water 0.1 ml was added to a vial containing 0.9 ml of plasma collected prior to drug administration. This yielded ciprofloxacin standards of 4, 2, 1, 0.5 and so on in plasma. From these three to four standards of ciprofloxacin was processed in appropriate procedure.

First blank plasma standards was injected in column of running, well stablised with baseline IIPLC instruments through rheodyne manual injector. After that three to four standards of enrofloxacin and ciprofloxacin were also injected in column of HPLC instruments through manual injector to confirm the peaks of enrofloxacin and its active metabolite ciprofloxacin at the particular retention time, in comparison with blank plasma standard.

Preparation of Mixed Standards of Enrofloxacin and Ciprofloxacin in Plasma :

From each standard solution of enrofloxacin in water 0.1 ml and from each standard solution of ciprofloxacin in water 0.1 ml was added to a clean tube/vial containing 0.8 ml of plasma collected prior to drug administration. This yielded drug standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 $\mu g\ ml^{-1}$ of both drugs in equal concentration in plasma. These standards were used simultaneously with test samples for HPLC analysis to estimate the drug concentration in test samples.

Analytical Method/Procedure :

1. In a clean and dry centrifuged tube 400 µl of plasma samples was taken and 600 µl of acetonitrile was added for precipitation of plasma proteins.
2. The mixture was shaken on a vortex mixer for 1 min. and centrifuged for 15 min at 3000 rpm.
3. Then, 300 µl of supernatant was transferred to a clean tube and mixed with 600 µl of triple distilled water.
4. An aliquot of this mixture (up to 400 µl) was injected directly into the loop of injector and the integrator recorded (print out) retention time and area.

5. From various concentrations of standards versus area, standard curve was plotted in a graph paper separately for enrofloxacin and ciprofloxacin. Using the standard graph, concentrations of test plasma samples collected at various time intervals were obtained from the area recorded by the integrator for the particular test sample.

Estimation of enrofloxacin and its active metabolite ciprofloxacin could not be standarised in urine samples since various constituents of urine interfered in the estimation as noted by Aerts *et al.*,1995 in HPLC method.

Hence the quantitative estimation of enrofloxacin (as well as active metabolite ciprofloxacin together) in urine samples was done by microbiological assay method (cylinder plate diffusion method).

2. **By Microbiological Assay Method** :

Procedure Adopted for the Microbiological Assay :

I. Sterilization of Glasswares, Needle and Porcelin Assay Cylinders :

All glasswares and procelin assay cylinders were washed properly with detergent solution in running tap water. These were again rinsed with glass distilled water and finally air-dried. Test tubes, centrifuge tubes, vials and vial containing procelin assay cylinders were plugged with cotton wool. Assay plates, pipettes and

syringes were wrapped with paper. All these materials were sterilized in hot air oven at 160°C for an hour. For administration of drug and for collection of blood, sterile disposable needles were used.

II. Preparation of Media :

(a) Assay Agar :

Antibiotic assay media of the following composition was used for microbiological assay of enrofloxacin in blood and urine after i.v. administration in buffalo calve.

S. No.	Ingredients	Grams/Litre Water
1.	Peptone	6.0
2.	Tryptone	4.0
3.	Yeast Extract	3.0
4.	Beef Extract	1.5
5.	Dextrose	1.0
6.	Agar	15.0
	Distilled Water	1000 ml
	Final pH	7.9 ± 0.1

The media was heated to dissolve and the solution was transferred into a conical flask, and pH was adjusted. The mouth of the flask was plugged with non-absorbable cotton wool and wrapped with aluminium foil. Wet sterilization of media was done by autoclaving at 15 pound pressure (121°C) for 20 minutes.

(b) Nutrient Broth :

Nutrient broth of the following composition was prepared :

Sl. No.	Ingredients	Grams/Litre Water
1.	Sodium chloride	5.0
2.	Peptone	10.0
3.	Beef Extract	10.0
	Distilled water	1000 ml.
	Final pH	7.4 ± 0.1

The media was heated to dissolve completely and pH was adjusted. Sterilization of the broth was done by autoclaving at 15 pound pressure (121°C) for 20 minutes.

III. Preparation of Assay Agar Plates :

Approx. 20 ml of autoclaved antibiotic assay media, while in melted condition, was poured gently into each of the sterilized special assay plate (Borosil) with the aid of a sterile measuring cylinder. The plates were kept on a horizontally plane surface to get uniform thickness of media. The plates were left at room temperature for about 1 to 2 h for solidification of agar. Afterwards the plates were kept inside the incubator at 37°C for 24 h to ascertain any growth, which indicates any microbial contamination. The growth free plates were then wrapped with sterile paper and stored in refrigerator until assay was carried out.

IV. Preparation of Test Organism :

The test organism used for the microbiological assay technique of enrofloxacin was *E. coli* (ATCC 25922). The culture of *E. coli* was obtained from National Collection of Industrial Micro-organism (NCIM), Division of Bio-chemical Sciences, National Chemical Laboratory, Poona - 8. The organism was grown on the slant of culture tube containing nutrient agar slants at 37°C for overnight. Then it was stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity.

V. Preparation of Standards in Urine :

Enrofloxacin was diluted in sterile glass distilled water to have different strengths viz. 40, 20, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 μg ml^{-1}. From each standard solution 0.1 ml was added to a sterile vial containing 0.9 ml of urine collected prior to drug administration. This yielded drug standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 μg ml^{-1} in the above noted biological fluid. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentrations in test samples.

VI. Assay Procedure :

Urine levels of enrofloxacin were estimated by microbiological assay technique (cylinder plate diffusion method) using *E. coli* (ATCC 25922) as the test organism. The test organism

was grown in nutrient broth for 1/2 to 1 hour at 37°C until the growth was seen (turbid by naked eye). Enrofloxacin assay plates were flooded with the broth containing the organism and excess broth was drained out after some time. The plates were dried in the incubator at 37°C for a period of about an hour. Sterile porcelin assay cylinders of uniform size were placed at appropriate distance along the circumference in the inoculated assay plates. 50 µl of standard solution of various strengths as well as test samples of the drug was poured in separate porcelin cylinder in the assay plate. Such plates were left on the table for about 2 hours and then kept in the incubator at 37°C for overnight in order to allow the growth of organism. The mean diameter of the bacterial zone of inhibition produced by the standards as well as test samples of the drug was measured. The concentrations of the drug in different test samples of urine were estimated from the standard curve plotted from the zone of inhibition versus concentration of the drug on a semilog scale.

Estimation of Diclofenac by Reverse Phase High Performance Liquid Chromatography (HPLC)) Method :

The concentrations of diclofenac sodium in plasma and urine were estimated by HPLC method as described by El-Sayed *et al.* (1988) with slight modification. The details of the procedure are as follows :

Apparatus :

The HPLC equipment used comprised of a HPLC pump, a dual wavelength absorbance detector, a rheodyne manual injector with a 20 µl loop size and a data module (integrator) as described earlier. Chromatographic separations were performed using C_{18} column (3.9 × 300 mm size) as noted above.

Chromatographic Conditions :

For HPLC analysis of diclofenac in biological samples, the flow rate was 1.5 ml. min^{-1}, the effluent was monitored at 280 nm, loop size was 20 µl, injection volume was 100 µl, chart speed was 0.25 mm. min^{-1} and the detector sensitivity was monitored at 2.000 A.U.F.S.

Reagents :

All solvents used were of HPLC grade. All other chemicals and reagents were of analytical grade and freshly prepared triple distilled water were used for HPLC analysis.

Mobile Phase :

The mobile phase comprised of acetonitrile : water (50 : 50% v/v), adjusted to pH 3.3 with glacial acetic acid.

Preparation of Standards of Diclofenac in Biological Samples :

Zobid®, an injectable commercial preparation containing diclofenac sodium in concentration of 25 mg. ml^{-1} was used in the

present study. Diclofenac was diluted in triple distilled water to have different strengths viz. 40, 20, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 $\mu g.ml^{-1}$.

From each standard solution 0.1 ml was added to a centrifuge tube containing 0.9 ml of plasma or urine collected prior to drug administration. This yielded diclofenac standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 $\mu g.ml^{-1}$ in the above noted biological fluid. Blank plasma / blank urine containing no drug was also prepared. These standards were used simultaneously with test samples for determination of the drug concentration in the test samples.

Analytical Method :

1. In a clean and dry centrifuged tube 1 ml of plasma samples was taken and 4 ml of acetonitrile was added for precipitation of plasma proteins.
2. The mixture was shaken on a vortex mixer for 1 min and centrifuged for 15 min at 3000 rpm.
3. The supernatent was transferred to a clean tube and evaporated to dryness in a boiling waterbath.
4. The residue is reconstituted in 400 µl HPLC eluent (mobile phase) and vortexed for 1 min.
5. An aliquot of this mixture (up to 100 µl) was injected directly into the loop of injector and the integrator print out retention time and area.

6. From various concentrations of standards versus area, standard curve was plotted in a graph paper for diclofenac.

7. Using these standard graph, the area obtained from test plasma and urine samples collected at various time intervals, the concentrations were obtained in test plasma and test urine samples separately.

CALCULATION OF PHARMACOKINETIC PARAMETERS

Pharmacokinetic parameters of enrofloxacin and diclofenac after a single i.v. administration were calculated from semilog plot of plasma drug concentration versus time curve. The experimental data was analysed using two compartment open model for enrofloxacin & diclofenac (i.v. route) and non-compartmental analysis for ciprofloxacin (active metabolite of enrofloxacin) as described by Gibaldi and Perrier, 1975 ; Notari, 1980 and Bhupinder Singh, 1999.

The concentration of the drug in plasma at any time is obtained by the following formulae :

$$C_p = A_e^{-\alpha t} + B_e^{-\beta t} \text{.....(Two compartment model)}$$

Where e is the base of natural logarithm and C_p is the drug concentration in plasma at time 't'. The description and calculation of the parameters A, B, α, and β used in the above formulae and other kinetic parameters are noted below :

(a) A, the zero time concentration of the drug in plasma and α, the regression coefficient (distribution rate constant) for distribution phase were calculated by the method of residual yields (Appendix 1).

(b) B, the zero time concentration of the drug in plasma and β, the regression coefficient (elimination rate constant), for elimination phase were calculated by the method of least squares (Appendix 1).

(c) C_p^0, the theoretical zero time plasma concentration of drug :

$$C_p^0 = A+B \text{ (Two compartment model)}$$

(d) Distribution half life ($t_{½}\ \alpha$) and elimination half life ($t_{½}\ \beta$) were calculated from the following formulae :

(i) For two compartment model.

$$t_{½}\ \alpha = 0.693/\alpha$$

$$t_{½}\ \beta = 0.693/\beta$$

α and β are described above.

(ii) For non-compartmental model

$$t_{½}\ \beta = 0.693 \times MRT$$

(e) AUC, the total area under plasma drug concentration time curve (mg. L^{-1}. h) :-

(i) For two compartment model

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

(ii) For non-compartmental model

Total AUC ($AUC_\infty = AUC_{t^*} + AUC_{t^*-\infty}$) [Bhupinder Singh, 1999]

where,

AUC_{t^*} = Area under curve up to last sampling.

$AUC_{t^*-\infty} = \frac{C^*}{\lambda}$ = Terminal AUC beyond last sampling.

C^* = last sampled plasma concentration.

λ = Slope of the terminal linear portion of log plasma concentration versus time curve.

(f) AUMC, the total area under the first moment of plasma drug concentration time curve (mg. L^{-1}. h^2) :

(i) For two compartment model

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

(ii) For non-compartmental model

Total AUMC ($AUMC_\infty = AUMC_{t^*} + AUMC_{t^*-\infty}$)

where,

$AUMC_{t^*}$ = Area under moment curve upto last sampling

$AUMC_{t^*-\infty} = C^*t^*/\lambda + (C^*/\lambda)^2$ = Terminal AUMC beyond last sampling.

C^* = last sampled plasma concentration.

t^* = last sampling time.

λ = Slope of the terminal linear portion of log plasma concentration versus time curve.

(g) MRT, mean residential time (h) :

(i) For two compartment model

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

(ii) For non-compartmental model

$$MRT = \frac{AUMC_{\infty}}{AUC_{\infty}}$$

(h) K_{21}, rate constant of transfer of drug from peripheral (tissue) compartment to the central (blood) compartment (h^{-1}) :

$$K_{21}, = \frac{A.\beta + B.\alpha}{C_p^0}$$

(i) Kel, the elimination rate constant of drug from central compartment (h^{-1}) :

$$Kel = \frac{\alpha.\beta}{K_{21}}$$

(j) K_{12}, the rate constant of transfer of drug from central to peripheral compartment (h^{-1}) :

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

(k) Fc, the fraction of drug available for elimination from central compartment :

$$\mathbf{Fc} = \frac{\beta}{Kel}$$

(l) T≈P, the approximate tissue to plasma concentration ratio:

$$T \approx P = \frac{K_{12}}{K_{21} - \beta}$$

(m) Vd_c, the volume of distribution, based on distribution and elimination ($L.kg^{-1}$) :

$$Vd_c = \frac{D}{C_p^0}$$

(n) Vd_B , the volume of distribution based on elimination ($L.kg^{-1}$) :

$$Vd_B = \frac{D}{B}$$

(o) Vd_{area}, the volume of distribution based on total area under curve ($L.kg^{-1}$) :

$$Vd_{area} = \frac{D}{AUC.\beta}$$

(p) Vd_{ss} , the volume of distribution at steady state ($L.kg^{-1}$) :

(i) For two compartment model

$$V_{SS} = \frac{K_{12} + K_{21}}{K_{21}} . Vd_C$$

(ii) For non-compartmental model

$$Vd_{SS} = \frac{Cl_B}{k \text{ or } \beta}$$

where, $k \text{ or } \beta = \frac{1}{MRT}$

(q) Cl_B, the total body clearance ($ml.kg^{-1}.min^{-1}$) :

(i) For two compartment model

$$Cl_B = Vd_{area} \times \beta$$

(ii) For non-compartmental model

$$Cl_B = \frac{X_0}{AUC_\infty}$$

where, X_0 = i.v. dose rate.

CALCULATION OF DOSAGE REGIMEN

Dosage regimen is generally calculated for an antimicrobial agent to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage intervals. The MIC values of enrofloxacin for different species of bacteria isolated from animals ranged between 0.001 to 1.0 μg. ml^{-1} (Mevius *et at.*,1990 ; Prescott and Yielding, 1990). The sensitivity or resistance of enrofloxacin is more or less similar to its close congener ciprofloxacin. The value of 0.12 $\mu g.ml^{-1}$ has been considered as MIC of ciprofloxacin for calculating dosage regimen by Raina (1991) and Singh *et al.* (2001). Hence, in the present study, dosage regimen of enrofloxacin was calculated at 0.125, 0.25 and 0.50 $\mu g.ml^{-1}$ levels for the dosage intervals of 8 and 12 h using the following formulae (Saini and Srivastava, 1997) :

$$D^* = C_p^{\infty}(\min).Vd_{area}\left(e^{\beta\gamma}\right)$$

$$D_0 = C_p^{\infty}(\min).Vd_{area}\left(e^{\beta\gamma} - 1\right)$$

where,

D^* = Loading or priming dose

D_0 = Maintenance dose

C_p^{∞} (min) = Desired minimum plasma concentration

γ = Dosage interval

e = Base of natural logarithm

β and Vd_{area} are obtained from kinetic study.

STATISTICAL ANALYSIS :

Comparison of concentrations of the drugs in plasma and urine at various time intervals, various kinetic parameters of the drugs and dosage regimen of enrofloxacin when the drugs were given alone and when given together in combination in buffalo calves were compared by using paired 't' test (Snedecor and Cochran, 1967).

Chapter – IV

Results

RESULTS

I. PHARMACOKINETIC STUDY AFTER A SINGLE INTRAVENOUS ADMINISTRATION

[A] Kinetic study of enrofloxacin :

The kinetic study of enrofloxacin and its active metabolite ciprofloxacin was estimated by HPLC method. However, concentrations of enrofloxacin (including its active metabolite ciprofloxacin) in urine were estimated by microbiological assay method.

ENROFLOXACIN

1. Plasma Levels :

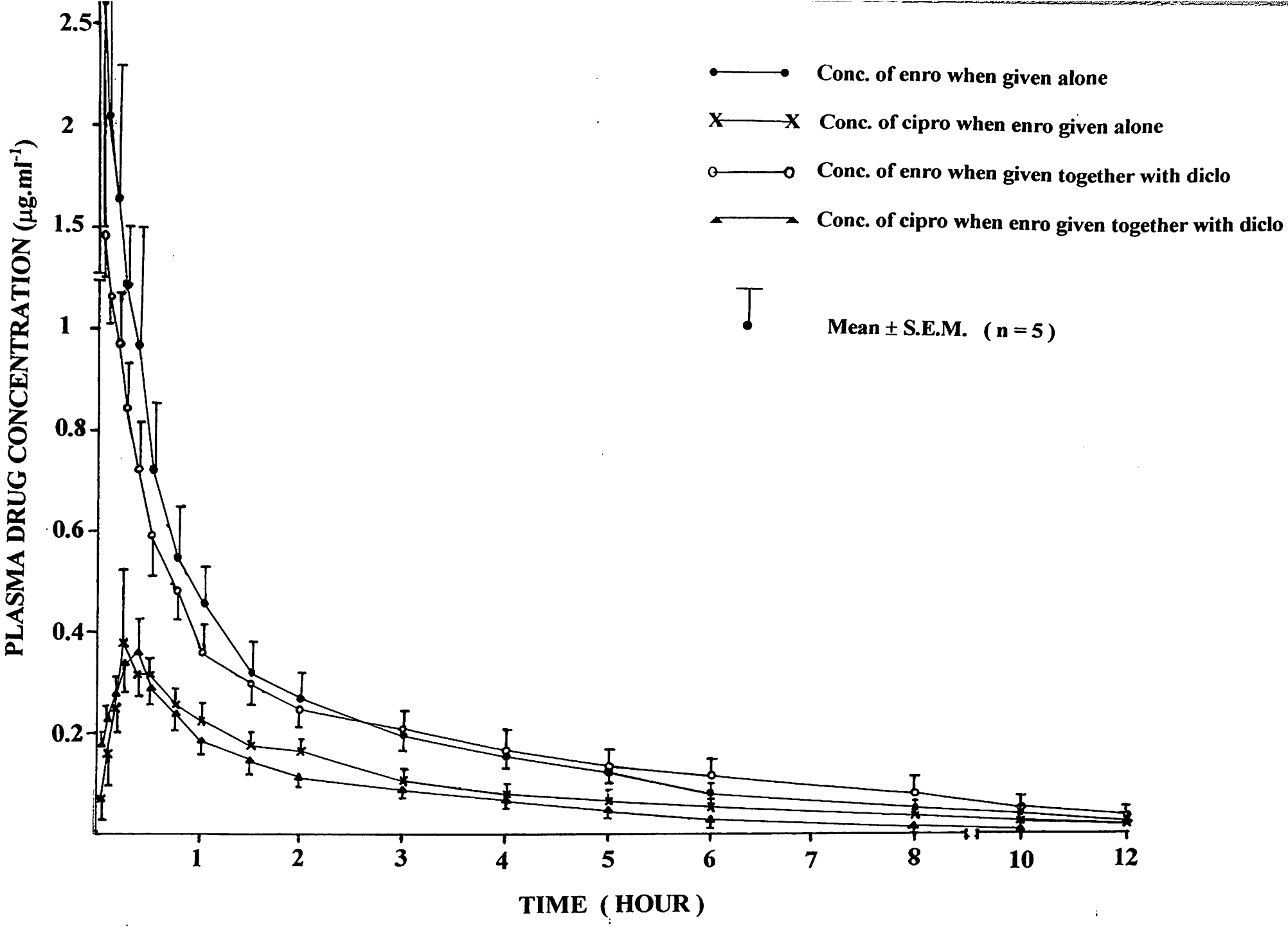
Concentrations of enrofloxacin in plasma at various time intervals following its single intravenous (i.v.) injection at the dose rate of 4 $mg.kg^{-1}$ body weight have been shown in Table 1 and Fig. 1. The mean plasma concentration of the drug at 0.042 h was found to be 2.61 ± 1.12 $\mu g.ml^{-1}$ and the value ranged from 1.32 to 7.10 $\mu g.ml^{-1}$. The drug was detectable in all the five animals upto 12 h and the mean concentration at 12 h was noted to be 0.02 ± 0.01 $\mu g.ml^{-1}$. The drug was not detectable in any of the animals at 24 h.

Table - 1

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin in buffalo calves following single intravenous dose of 4 $mg.kg^{-1}$.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	7.10	1.64	1.32	1.42	1.55	2.61 ±1.12
0.083	5.34	1.19	1.21	1.23	1.28	2.05 ±0.82
0.167	3.74	0.97	0.98	1.05	1.07	1.56 ±0.54
0.25	2.38	0.86	0.88	0.94	0.93	1.20 ±0.30
0.333	1.84	0.61	0.64	0.86	0.89	0.97 ±0.23
0.50	1.19	0.41	0.49	0.71	0.75	0.72 ±0.13
0.75	0.89	0.33	0.36	0.56	0.62	0.55 ±0.10
1	0.70	0.30	0.32	0.46	0.53	0.46 ±0.07
1.5	0.46	0.18	0.20	0.30	0.46	0.32 ± 0.06
2	0.38	0.17	0.17	0.26	0.38	0.27 ±0.05
3	0.20	0.16	0.13	0.20	0.29	0.20 ±0.03
4	0.16	0.15	0.10	0.17	0.23	0.16 ±0.02
5	0.13	0.14	0.08	0.13	0.18	0.13 ±0.02
6	0.10	0.03	0.06	0.09	0.13	0.08 ±0.02
8	0.07	0.02	0.04	0.06	0.08	0.05 ±0.01
10	0.06	0.01	0.02	0.04	0.05	0.04 ±0.01
12	0.05	0.01	0.01	0.02	0.03	0.02 ± 0.01
24	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D = Non – detectable


PLASMA DRUG CONCENTRATION (μg.ml-1)
2.5
2
1.5
1
0.8
0.6
0.4
0.2
1
2
3
4
5
6
7
8
10
12
TIME (HOUR)
Conc. of enro when given alone
Conc. of cipro when enro given alone
Conc. of enro when given together with diclo
Conc. of cipro when enro given together with diclo
Mean ± S.E.M. (n = 5)

2. *Kinetic Parameters :*

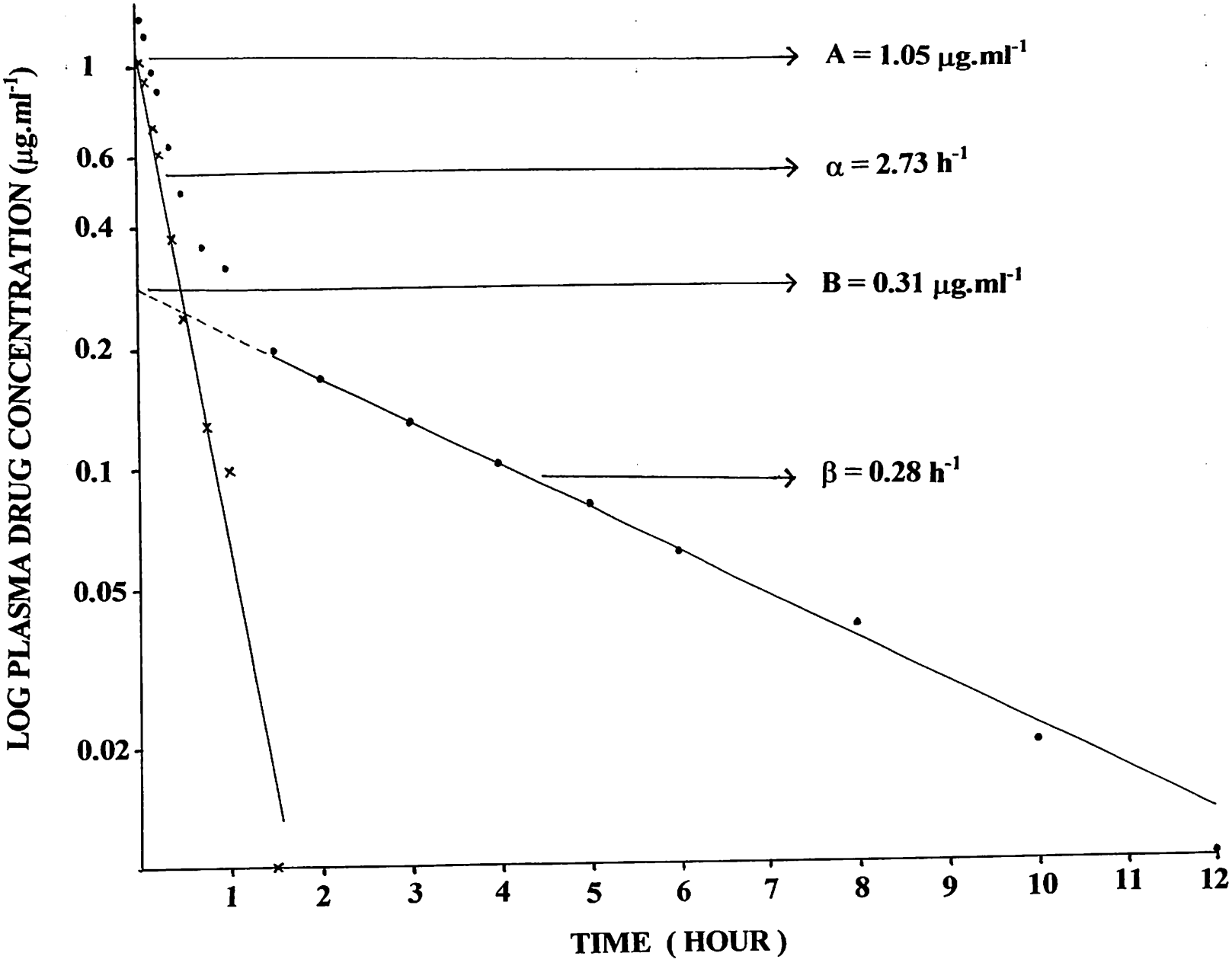
Plasma drug concentration versus time profile has confirmed the two compartment open model for enrofloxacin as depicted in Fig. 2. Table 2 shows the value of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration ($C_p^0 = A + B$) were noted to be 1.56 ± 0.58, 0.41 ± 0.06 and 1.97 ± 0.55 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.83 to 4.18 h^{-1} with a mean value of 2.65 ± 0.41 h^{-1} while its elimination rate constant (β) ranged from 0.16 to 0.33 h^{-1} with a mean value of 0.25 ± 0.03 h^{-1}. The mean distribution half-life ($t_{½}\ \alpha$) and elimination half-life ($t_{½}\ \beta$) were calculated to be 0.28 ± 0.04 and 2.92 ± 0.41 h, respectively. The mean area under curve in plasma (AUC) and mean area under first moment curve (AUMC) were noted to be 2.37 ± 0.45 mg. $L^{-1}.h$ and 7.44 ± 1.67 $mg.L^{-1}.h^2$, respectively, with the mean residential time (MRT) of 3.05 ± 0.20 h. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.17 ± 0.27, 1.06 ± 0.09 and 0.68 ± 0.15 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate

Table - 2

Kinetic parameters of enrofloxacin in buffalo calves calculated by 2-compartment open model following single intravenous dose of 4 mg.kg^{-1}.

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A(μg.ml^{-1})	3.87	1.23	1.05	0.95	0.72	1.56 ± 0.58
B(μg.ml^{-1})	0.28	0.38	0.31	0.44	0.63	0.41 ± 0.06
C_p^0(μg.ml^{-1})	4.15	1.61	1.36	1.39	1.35	1.97 ± 0.55
α (h^{-1})	1.83	4.18	2.73	2.10	2.42	2.65 ± 0.41
$t_{½}$ α (h)	0.38	0.17	0.25	0.33	0.29	0.28 ± 0.04
β (h^{-1})	0.16	0.33	0.28	0.25	0.25	0.25 ± 0.03
$t_{½}$ β (h)	4.46	2.10	2.48	2.77	2.77	2.92 ± 0.41
AUC (mg.L^{-1}.h)	3.86	1.45	1.49	2.21	2.82	2.37 ± 0.45
AUMC (mg.L^{-1}.h^2)	12.09	3.56	4.09	7.26	10.20	7.44 ± 1.67
MRT (h)	3.13	2.46	2.74	3.29	3.62	3.05 ± 0.20
K_{12}(h^{-1})	0.60	2.16	1.26	0.89	0.93	1.17 ± 0.27
K_{21}(h^{-1})	1.13	1.24	0.84	0.84	1.26	1.06 ± 0.09
Kel (h^{-1})	0.26	1.11	0.91	0.63	0.48	0.68 ± 0.15
Fc	0.62	0.30	0.31	0.40	0.52	0.43 ± 0.06
T≈P	0.62	2.37	2.25	1.50	0.92	1.53 ± 0.35
Vdc (L.kg^{-1})	0.96	2.48	2.94	2.88	2.96	2.44 ± 0.38
Vd_B (L.kg^{-1})	14.29	10.53	12.90	9.09	6.35	10.63 ± 1.40
Vd_{area} (L.kg^{-1})	6.48	8.36	9.59	7.24	5.67	7.47 ± 0.69
Vd_{SS} (L.kg^{-1})	1.47	6.80	7.35	5.91	5.14	5.33 ± 1.04
Cl_B(ml.kg^{-1}.min^{-1})	17.33	46.00	44.83	30.17	23.67	32.40 ± 5.69


LOG PLASMA DRUG CONCENTRATION (μg.ml-1)
1
0.6
0.4
0.2
0.1
0.05
0.02
A = 1.05 μg.ml-1
α = 2.73 h-1
B = 0.31 μg.ml-1
β = 0.28 h-1
1
2
3
4
5
6
7
8
9
10
11
12
TIME (HOUR)

tissue to plasma concentration ratio (T≈P) were noted to be 0.43 ± 0.06 and 1.53 ± 0.35, respectively. The various values of volume of distribution calculated by different methods are shown in Table 2. The mean volume of distribution (Vd_{area}) was calculated to be 7.47 ± 0.69 $L.kg^{-1}$. The total body clearance (Cl_R) ranged from 17.33 to 46.00 with a mean of 32.40 ± 5.69 $ml.kg^{-1}.min^{-1}$.

CIPROFLOXACIN

1. Plasma Levels :

Plasma concentrations of ciprofloxacin (active metabolite of enrofloxacin) at various time intervals following a single i.v. injection of enrofloxacin (4 $mg.kg^{-1}$) have been shown in Table 3 and Fig 1. The mean plasma concentration of ciprofloxacin at 0.042 h was found to be 0.07 ± 0.04 $\mu g.ml^{-1}$). Peak concentration of the drug was attained at 0.25 h (0.38 ± 0.14 $\mu g.ml^{-1}$). The drug was detectable in four out of five animals at 10 h and two out of five animals at 12 h and the mean concentration at 12 h was noted to be 0.02 ± 0.01 $\mu g.ml^{-1}$. The drug was not detectable in any of the animals at 24 h.

2. Kinetic Parameters :

Plasma drug concentration versus time profile had shown non-linear pattern and hence, it may best be described by non-compartmental model. Table 4 shows the values of different kinetic parameters calculated by the non-compartmental analysis.

Table - 3

Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin in buffalo calves following single intravenous dose of enrofloxacin (4 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	0.21	N.D.	0.03	0.04	0.09	0.07 ± 0.04
0.083	0.36	0.03	0.11	0.16	0.12	0.16 ± 0.06
0.167	0.41	0.17	0.24	0.26	0.18	0.25 ± 0.04
0.25	0.92	0.20	0.25	0.33	0.22	0.38 ± 0.14
0.333	1.46	0.23	0.28	0.37	0.24	0.32 ± 0.04
0.50	0.43	0.26	0.31	0.31	0.28	0.32 ± 0.03
0.75	0.39	0.20	0.23	0.26	0.22	0.26 ± 0.03
1	0.35	0.19	0.18	0.22	0.19	0.23 ± 0.03
1.5	0.27	0.15	0.15	0.19	0.16	0.18 ± 0.02
2	0.26	0.14	0.13	0.16	0.13	0.16 ± 0.02
3	0.20	0.10	0.05	0.12	0.09	0.11 ± 0.02
4	0.14	0.07	0.03	0.10	0.06	0.08 ± 0.02
5	0.13	0.06	0.03	0.09	0.05	0.07 ± 0.02
6	0.12	0.05	0.02	0.07	0.04	0.06 ± 0.02
8	0.08	0.03	0.01	0.05	0.02	0.04 ± 0.01
10	0.07	0.01	N.D.	0.04	0.01	0.03 ± 0.01
12	0.06	N.D.	-	0.02	N.D.	0.02 ± 0.01
24	N.D.	-	-	N.D.	-	-

N.D. = Non – detectable

Table – 4

Kinetic parameters of ciprofloxacin in buffalo calves calculated by non-compartmental analysis following single intravenous dose of enrofloxacin (4 mg.kg^{-1}).

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
K or β (h^{-1})	0.21	0.30	0.47	0.24	0.34	0.31 ± 0.05
$t_{½}$ β (h)	3.31	2.28	1.46	2.92	2.05	2.40 ± 0.33
AUC ($mg.L^{-1}.h$)	2.09	0.82	0.62	1.22	0.77	1.10 ± 0.27
AUMC ($mg.L^{-1}.h^2$)	10.00	2.70	1.31	5.14	2.28	4.29 ± 1.56
MRT (h)	4.78	3.29	2.11	4.21	2.96	3.47 ± 0.47
Vd_{SS} ($L.kg^{-1}$)	9.09	16.27	13.72	13.67	15.26	13.60 ± 1.23
Cl_B ($ml.kg^{-1}.min^{-1}$)	31.90	81.30	107.53	54.67	86.50	72.38 ± 13.17
% Conversion of enrofloxacin to ciprofloxacin $\left(\frac{\text{AUC cipro}}{\text{AUC enro}}\right)$	54.15	56.55	41.61	55.20	27.30	46.96 ± 5.60

The elimination rate constant (k or β) ranged from 0.21 to 0.47 h^{-1} with a mean value of 0.31 ± 0.05 h^{-1} while the elimination half-life ($t_{½}$ β) ranged from 1.46 to 3.31 h with a mean value of 2.40 ± 0.33 h. The mean area under curve in plasma (AUC) and area under first moment curve (AUMC) were noted to be 1.10 ± 0.27 mg. $L^{-1}.h$ and 4.29 ± 1.56 mg. $L^{-1}.h^2$ with the mean residential time (MRT) of 3.47 ± 0.47 h. The mean value of volume of distribution at steady state (Vd_{SS}) was calculated to be 13.60 ± 1.23 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 31.90 to 107.53 with a mean of 72.38 ± 13.17 $ml.kg^{-1}.min^{-1}$. The percentage conversion of enrofloxacin to ciprofloxacin ranged from 27.30 to 56.55 with a mean of 46.96 ± 5.60.

ENROFLOXACIN + CIPROFLOXACIN

1. Plasma Levels :

Plasma concentrations of enrofloxacin + ciprofloxacin (active metabolite of enrofloxacin) together in buffalo calves following single intravenous dose of enrofloxacin (4$mg.kg^{-1}$) has been shown in Table 5. The mean plasma concentration of the drug at 0.042 h was found to be 2.68 ± 1.16 $\mu g.ml^{-1}$ and the value ranged from 1.35 to 7.31 $\mu g.ml^{-1}$. The drug was detectable in all five animals at 12 h and the mean plasma concentration was 0.04 ± 0.02 $\mu g.ml^{-1}$. The drug was not detected at 24 h in any of the buffalo calves. The minimum therapeutic concentration (≥ 0.125 $\mu g.ml^{-1}$) was maintained upto 6 h.

Table - 5

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin+ ciprofloxacin together in buffalo calves following single intravenous dose of enrofloxacin (4 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	7.31	1.64	1.35	1.46	1.64	2.68 ± 1.16
0.083	5.70	1.22	1.32	1.39	1.40	2.21 ± 0.87
0.167	4.15	1.14	1.22	1.31	1.25	1.81 ± 0.58
0.25	3.30	1.06	1.13	1.27	1.15	1.58 ± 0.43
0.333	2.30	0.84	0.92	1.23	1.13	1.28 ± 0.26
0.50	1.62	0.72	0.80	1.02	1.03	1.04 ± 0.16
0.75	1.28	0.53	0.59	0.82	0.84	0.81 ±0.13
1	1.05	0.49	0.50	0.68	0.72	0.69 ± 0.10
1.5	0.73	0.33	0.35	0.49	0.62	0.50 ± 0.08
2	0.64	0.31	0.30	0.42	0.51	0.44 ± 0.06
3	0.40	0.26	0.18	0.32	0.38	0.31 ± 0.04
4	0.30	0.22	0.13	0.27	0.29	0.24 ± 0.03
5	0.26	0.20	0.11	0.22	0.23	0.20 ± 0.03
6	0.22	0.08	0.08	0.16	0.17	0.14 ± 0.03
8	0.15	0.05	0.05	0.11	0.10	0.09 ± 0.02
10	0.13	0.02	0.02	0.08	0.06	0.06 ±0.02
12	0.11	0.01	0.01	0.04	0.03	0.04 ± 0.02
24	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non - detectable

2. *Kinetic Parameters :*

Plasma drug concentration versus time profile has confirmed the two-compartment open model. Table 6 shows the values of important kinetic parameters of enrofloxacin+ciprofloxacin together needed for calculation of dosage regimen of enrofloxacin in buffalo calves calculated by the above noted compartment model.

The mean extrapolated zero time concentration of enrofloxacin + ciprofloxacin together in plasma during distribution phase (A) and elimination phase (B) were noted to be 1.56 ± 0.66 and 0.66 ± 0.07 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.70 to 4.06 h^{-1} with a mean value of 2.47 ± 0.42 h^{-1} while its elimination rate constant (β) ranged from 0.14 to 0.35 h^{-1} with a mean value of 0.26 ± 0.04 h^{-1}. The mean distribution half life ($t_{½}\ \alpha$) and elimination half-life ($t_{½}\ \beta$) were observed to be 0.31 ± 0.04 and 2.93 ± 0.53 h, respectively. The value of area under curve in plasma (AUC) was found to be 3.52 ± 0.76 mg. L^{-1}.h. The mean value of Vd_{area} was calculated to be 4.98 ± 0.33 $L.kg^{-1}$.

3. *Dosage Regimen :*

The dosage regimen required to maintain the different levels of therapeutic concentration (C_p^{∞} min = 0.125, 0.25 and 0.50 $\mu g.ml^{-1}$) in plasma for i.v. route in buffalo calves at different dosage intervals (γ) of 8 and 12 h is presented in Table 7. For maintaining

Table – 6

Important kinetic parameters of enrofloxacin+ciprofloxacin together needed for calculation of dosage regimen of enrofloxacin calculated by 2-compartment open model following single i.v.dose of enrofloxacin(4 mg.kg^{-1}).

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A (μg. ml^{-1})	4.20	0.88	0.92	1.15	0.65	1.56 ± 0.66
B (μg. ml^{-1})	0.54	0.73	0.51	0.65	0.89	0.66 ± 0.07
α (h^{-1})	1.70	4.06	1.93	2.37	2.27	2.47 ± 0.42
$t_{½}$ α(h)	0.41	0.17	0.36	0.29	0.31	0.31 ± 0.04
β (h^{-1})	0.14	0.35	0.32	0.22	0.28	0.26 ± 0.04
$t_{½}$ β(h)	4.91	1.99	2.17	3.09	2.50	2.93 ± 0.53
AUC (mg. L^{-1}. h)	6.33	2.30	2.07	3.44	3.46	3.52 ± 0.76
Vd_{area}(L.kg^{-1})	4.48	4.96	6.04	5.29	4.13	4.98 ± 0.33

Table – 7

Dosage regimen of enrofloxacin for intravenous route in buffalo calves.

C_p^{∞} min ($\mu g.ml^{-1}$)	γ(h)	Dose ($mg.kg^{-1}$)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
0.125	8	D*	1.72	10.20	9.76	3.84	4.85	6.07 ± 1.67
		D_0	1.16	9.58	9.01	3.18	4.33	5.45 ± 1.65
	12	D*	3.00	41.34	35.12	9.27	14.86	20.72 ± 7.46
		D_0	2.44	40.72	34.37	8.60	14.35	20.10 ± 7.44
0.25	8	D*	3.44	20.40	19.52	7.68	9.70	12.15 ± 3.35
		D_0	2.32	19.16	18.02	6.36	8.66	10.90 ± 3.30
	12	D*	6.00	82.68	70.24	18.54	29.72	41.44 ± 14.92
		D_0	4.88	81.44	68.74	17.20	28.70	40.20 ± 14.88
0.50	8	D*	6.88	40.80	39.04	15.36	19.40	24.30 ± 6.70
		D_0	4.64	38.32	36.04	12.72	17.32	21.80 ± 6.60
	12	D*	12.00	165.4	140.5	37.08	59.44	82.88 ± 29.84
		D_0	9.76	162.9	137.5	34.40	57.40	80.40 ± 29.76

D* = Priming or Loading dose

D_0 = Maintenance dose

γ = Dosage interval

C_p^{∞} min = Minimum therapeutic concentration in plasma (MIC).

C_p^{∞} min of 0.125 μg.ml^{-1}, the loading doses (D*) were calculated to be 6.07 ± 1.67 and 20.72 ± 7.46 mg.kg^{-1} while maintenance doses (D_0) were calculated to be 5.45 ± 1.65 and 20.10 ± 7.44 mg.kg^{-1} at the dosage intervals (γ) of 8 and 12 h, respectively. The D*s were calculated to be 12.15 ± 3.35 and 41.44 ± 14.92 mg.kg^{-1} while D_0s were found to be 10.90 ± 3.30 and 40.20 ± 14.88 mg.kg^{-1} at γ of 8 and 12 h, respectively, for maintaining C_p^{∞} min of 0.25 μg.ml^{-1}. Like-wise, to maintain C_p^{∞} min of 0.50 μg.ml^{-1} the D*s were calculated to be 24.30 ± 6.70 and 82.88 ± 29.84 mg.kg^{-1} while D_0s were found to be 21.80 ± 6.60 and 80.40 ± 29.76 mg.kg^{-1} at γ of 8 and 12 h, respectively.

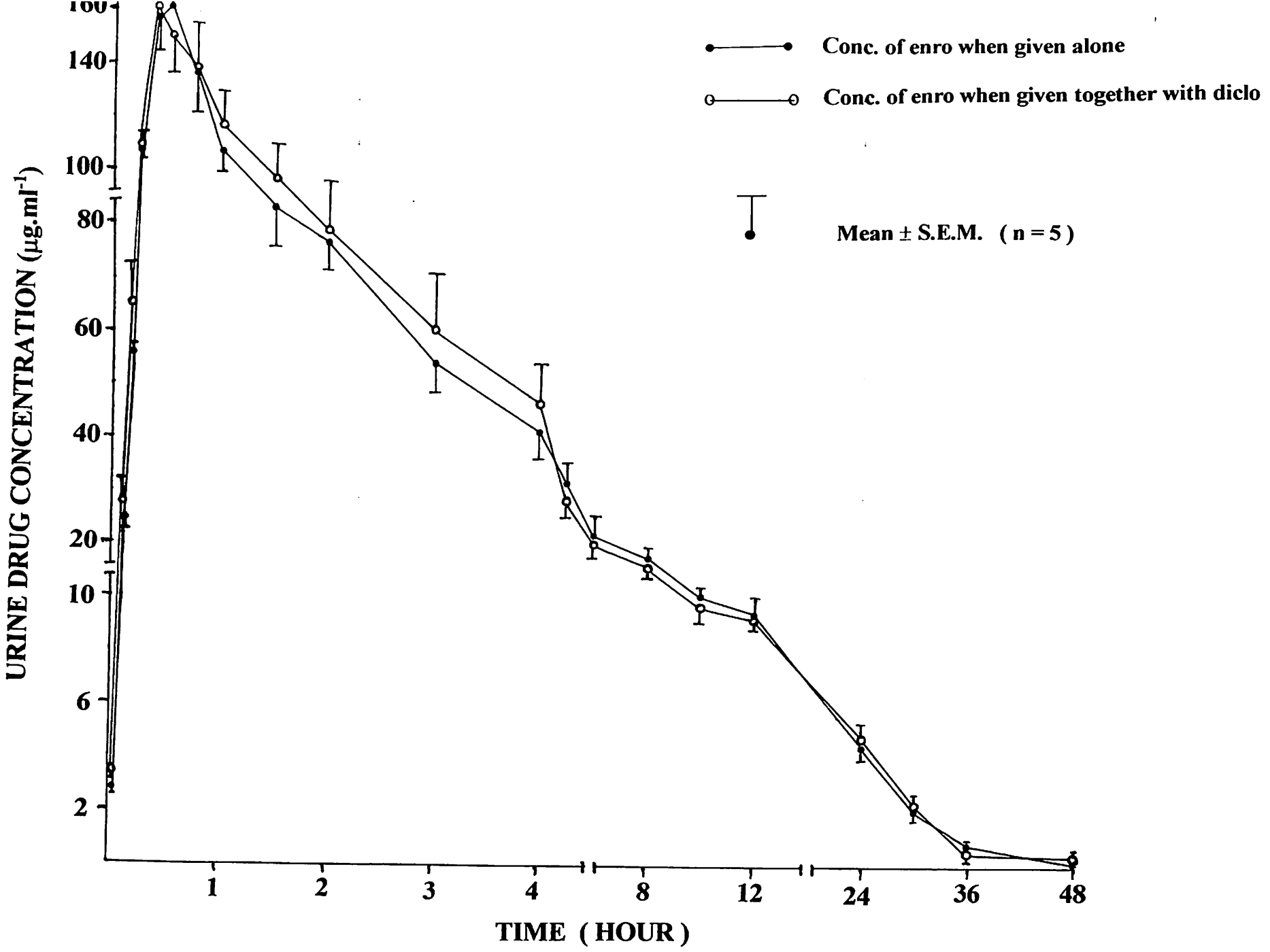
4. ***Urine Levels :***

Concentrations of enrofloxacin (including its active metabolite ciprofloxacin) in urine estimated by microbiological assay in buffalo calves post i.v. administration of enrofloxacin (4 mg.kg^{-1}) have been depicted in Table 8 and Fig. 3. The drug appeared in effective therapeutic concentration (≥ 0.125 μg.ml^{-1}) in all five animals at 0.042 h and was maintained even beyond 48 h. The mean peak urine concentration of 161.6 ± 12.20 μg.ml^{-1} was observed at 0.50 h. The drug was detectable in all five animals at 48 h (0.23 ± 0.03 μg.ml^{-1}).

Table - 8

Urine concentrations ($\mu g.ml^{-1}$) of enrofloxacin (including its active metabolite ciprofloxacin) estimated by microbiological assay in buffalo calves following single intravenous dose of 4 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	2.84	2.63	2.97	3.21	2.83	2.90 ± 0.10
0.083	21.23	22.42	29.23	26.12	25.69	24.94 ± 1.42
0.167	53.93	55.18	58.92	59.23	56.24	56.70 ±1.04
0.25	101.7	106.2	107.7	111.2	109.6	107.3 ± 1.63
0.333	132.5	190.6	186.2	145.3	136.1	158.1± 12.55
0.50	186.9	132.7	135.2	189.6	163.4	161.6 ± 12.20
0.75	135.2	105.9	107.3	151.4	188.7	137.7 ± 15.40
1	118.6	91.24	95.20	106.7	122.2	106.8 ± 6.14
1.5	103.2	68.92	71.32	92.34	97.32	86.62 ± 6.96
2	90.31	63.12	67.12	81.24	82.49	76.86 ±5.08
3	71.21	42.31	47.21	53.12	56.31	54.03 ± 4.92
4	60.23	33.12	33.69	40.32	45.28	42.53 ± 4.96
5	45.32	25.42	26.71	32.93	33.61	32.80 ± 3.53
6	32.13	18.10	19.35	26.12	19.21	22.98 ± 2.69
8	24.21	15.72	18.01	19.63	16.12	18.74 ± 1.54
10	17.12	8.89	10.21	13.21	9.32	11.75 ± 1.54
12	9.23	6.32	8.31	7.32	6.79	7.59 ± 0.53
24	6.01	3.93	5.23	4.39	3.82	4.68 ± 0.42
30	3.12	1.96	2.50	2.01	1.81	2.28 ± 0.24
36	0.91	0.86	1.01	0.82	0.85	0.89 ± 0.03
48	0.34	0.19	0.23	0.16	0.21	0.23 ± 0.03


Conc. of enro when given alone
Conc. of enro when given together with diclo
Mean ± S.E.M. (n = 5)
URINE DRUG CONCENTRATION (μg.ml-1)
160
140
100
80
60
40
20
10
6
2
1
2
3
4
8
12
24
36
48
TIME (HOUR)

[B] Kinetic study of diclofenac :

The kinetic study of diclofenac in buffalo calves after a single intravenous administration was estimated by HPLC method.

1. Plasma levels :

Plasma concentrations of diclofenac at various time intervals following single intravenous dose of 1 $mg.kg^{-1}$ in buffalo calves have been shown in Table 9 and Fig. 4. The mean plasma concentration of the drug at 0.042 h was found to be 7.04 ± 0.75 $\mu g.ml^{-1}$ and the value ranged from 4.21 to 8.73 $\mu g.ml^{-1}$. The drug was detectable in three out of five animals at 24 h and the mean plasma concentration was 0.03 ± 0.01 $\mu g.ml^{-1}$.

2. Kinetic parameters :

Plasma drug concentration versus time profile has confirmed the two-compartment open model. Table 10 shows the values of different kinetic parameters calculated by the above noted compartment model.

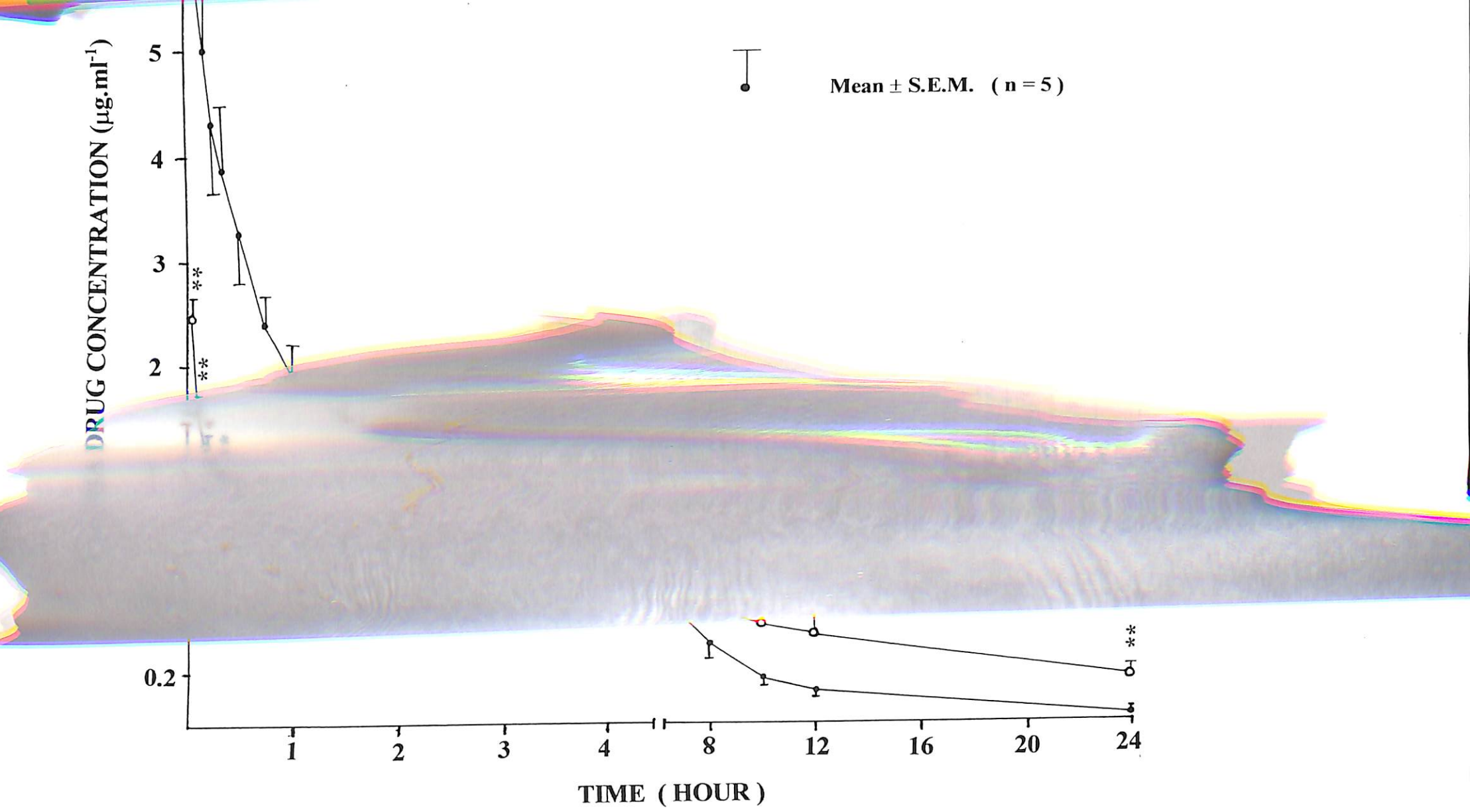
The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and theoretical zero time concentration ($C_p^0 = A+B$) were noted to be 5.74 ± 1.20, 1.65 ± 0.35 and 7.38 ± 1.49 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.18 to 5.70 h^{-1} with a mean value of 2.76 ± 0.81 h^{-1} while its elimination rate constant (β)

Table – 9

Plasma concentrations ($\mu g.ml^{-1}$) of diclofenac in buffalo calves following single intravenous dose of 1 $mg.kg^{-1}$.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	4.21	8.73	7.63	7.25	7.40	7.04 ± 0.75
0.083	3.02	6.81	6.16	6.20	6.25	5.69 ± 0.68
0.167	1.69	6.47	5.99	5.42	5.45	5.00 ± 0.85
0.25	1.67	5.23	4.75	5.00	5.00	4.33 ± 0.67
0.333	1.50	4.65	4.51	4.32	4.35	3.87 ± 0.59
0.50	1.49	4.06	3.56	3.84	3.42	3.27 ± 0.46
0.75	1.46	3.01	2.54	2.62	2.45	2.42 ± 0.26
1	1.10	2.59	2.08	2.00	1.90	1.93 ± 0.24
1.5	0.93	1.82	1.72	1.50	1.60	1.51 ± 0.16
2	0.89	1.48	1.61	1.35	1.25	1.32 ± 0.12
3	0.71	0.79	1.23	1.15	1.00	0.98 ± 0.10
4	0.69	0.59	1.03	0.90	0.72	0.79 ± 0.08
5	0.56	0.55	0.77	0.70	0.58	0.63 ± 0.04
6	0.42	0.47	0.56	0.65	0.50	0.52 ± 0.04
8	0.36	0.26	0.25	0.50	0.24	0.32 ± 0.05
10	0.30	0.10	0.17	0.22	0.18	0.19 ± 0.03
12	0.20	0.09	0.13	0.12	0.15	0.14 ± 0.02
24	0.06	0.02	N.D.	N.D.	0.07	0.03 ± 0.01

N.D. = Non – detectable


DRUG CONCENTRATION (μg.ml-1)
5
4
3
2
0.2
**
**
**
Mean ± S.E.M. (n = 5)
1
2
3
4
8
12
16
20
24
TIME (HOUR)

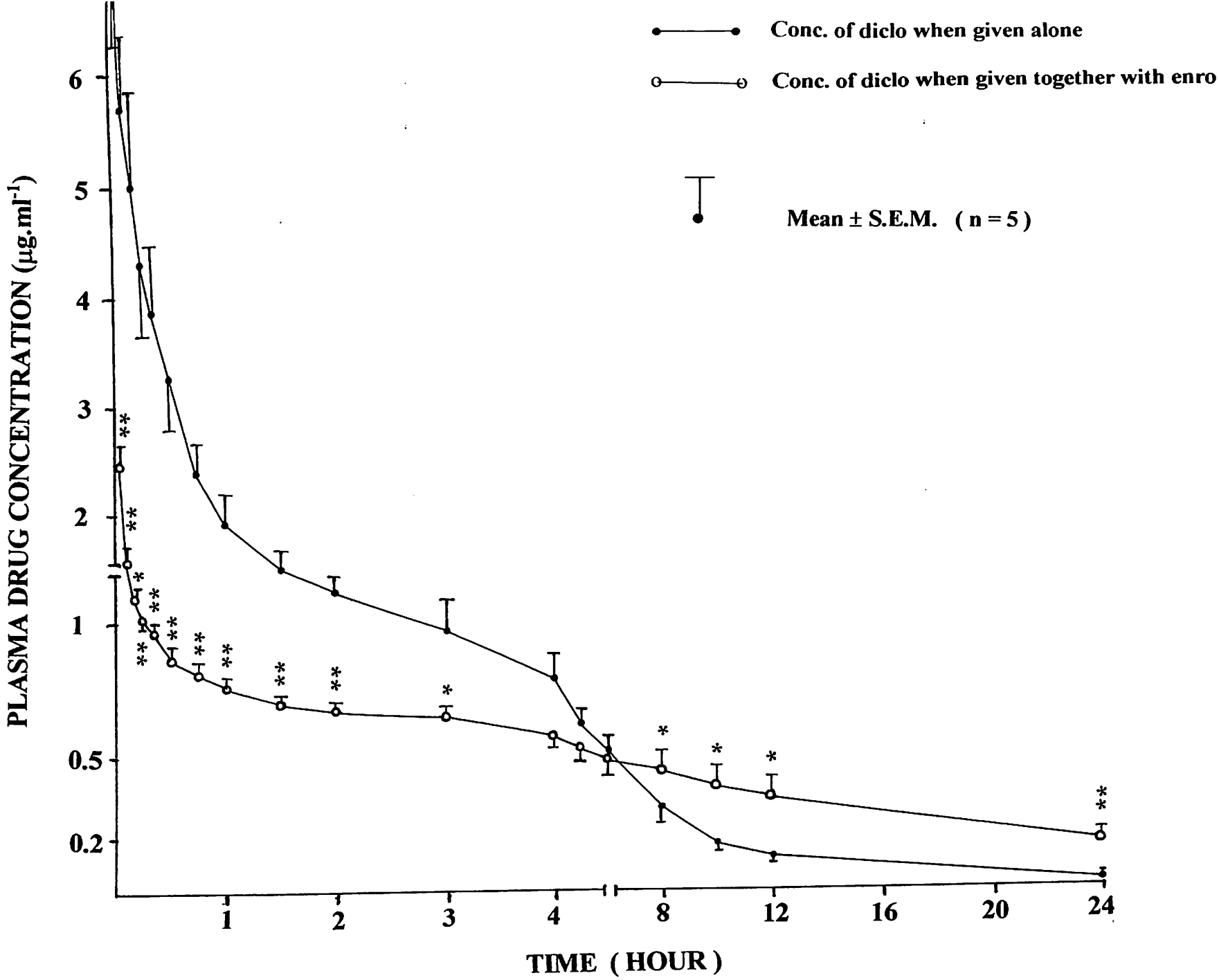

Conc. of diclo when given alone
Conc. of diclo when given together with enro
Mean ± S.E.M. (n = 5)
PLASMA DRUG CONCENTRATION (μg.ml-1)
6
5
4
3
2
1
0.5
0.2
1
2
3
4
8
12
16
20
24
TIME (HOUR)

Table – 10

Kinetic parameters of diclofenac in buffalo calves following single intravenous dose of 1 mg.kg^{-1}.

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A (μg. ml^{-1})	1.70	5.98	9.27	6.07	5.66	5.74 ± 1.20
B (μg. ml^{-1})	1.03	1.12	2.71	2.29	1.09	1.65 ± 0.35
C_p^0 (μg. ml^{-1})	2.73	7.10	11.98	8.36	6.75	7.38 ± 1.49
α (h^{-1})	2.19	1.18	5.70	3.18	1.56	2.76 ± 0.81
$t_{½}$ α (h)	0.32	0.59	0.12	0.22	0.44	0.34 ± 0.08
β (h^{-1})	0.12	0.18	0.27	0.23	0.13	0.19 ± 0.03
$t_{½}$ β (h)	5.62	3.84	2.59	3.02	5.22	4.06 ± 0.59
AUC (mg.L^{-1}.h)	9.36	11.29	11.66	11.87	12.01	11.24 ± 0.48
AUMC (mg. L^{-1}.h^2)	71.88	38.86	37.46	43.89	66.82	51.78 ± 7.30
MRT (h)	7.68	3.44	3.21	3.70	5.56	4.72 ± 0.85
K_{12} (h^{-1})	1.12	0.40	3.44	1.67	0.77	1.48 ± 0.53
K_{21} (h^{-1})	0.90	0.34	1.50	1.04	0.36	0.83 ± 0.22
Kel (h^{-1})	0.29	0.62	1.03	0.70	0.56	0.64 ± 0.12
Fc	0.41	0.29	0.26	0.33	0.23	0.30 ± 0.03
T ≈ P	1.44	2.50	2.80	2.06	3.35	2.43± 0.32
Vdc (L.kg^{-1})	0.37	0.14	0.08	0.12	0.15	0.17 ± 0.05
Vd_B (L.kg^{-1})	0.97	0.89	0.37	0.44	0.92	0.72 ± 0.13
Vd_{area} (L.kg^{-1})	0.89	0.49	0.32	0.37	0.64	0.54 ± 0.10
Vd_{ss} (L.kg^{-1})	0.83	0.30	0.26	0.31	0.47	0.43 ± 0.10
Cl_B (ml. kg^{-1}.min^{-1})	1.78	1.50	1.50	1.50	1.34	1.52 ± 0.07

ranged from 0.12 to 0.27 h^{-1} with a mean value of 0.19± 0.03 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) values of the drug were observed to be 0.34±0.08 and 4.06 ± 0.59 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.48 ± 0.53, 0.83 ±0.22 and 0.64 ± 0.12 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.30 ± 0.03 and 2.43 ± 0.32 . The value of area under curve in plasma (AUC) and area under first moment curve (AUMC) were found to 11.24 ± 0.48 $mg.L^{-1}.h$ and 51.78 ± 7.30 $mg.L^{-1}.h^2$ with a mean residential time (MRT) of 4.72 ± 0.85 h. The various values of volume of distribution calculated by different methods are show in Table 10. The mean value of Vd_{area} was calculated to be 0.54 ± 0.10 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 1.34 to 1.78 with a mean value of 1.52 ± 0.07 $ml.kg^{-1}.min^{-1}$.

3. Urine levels :

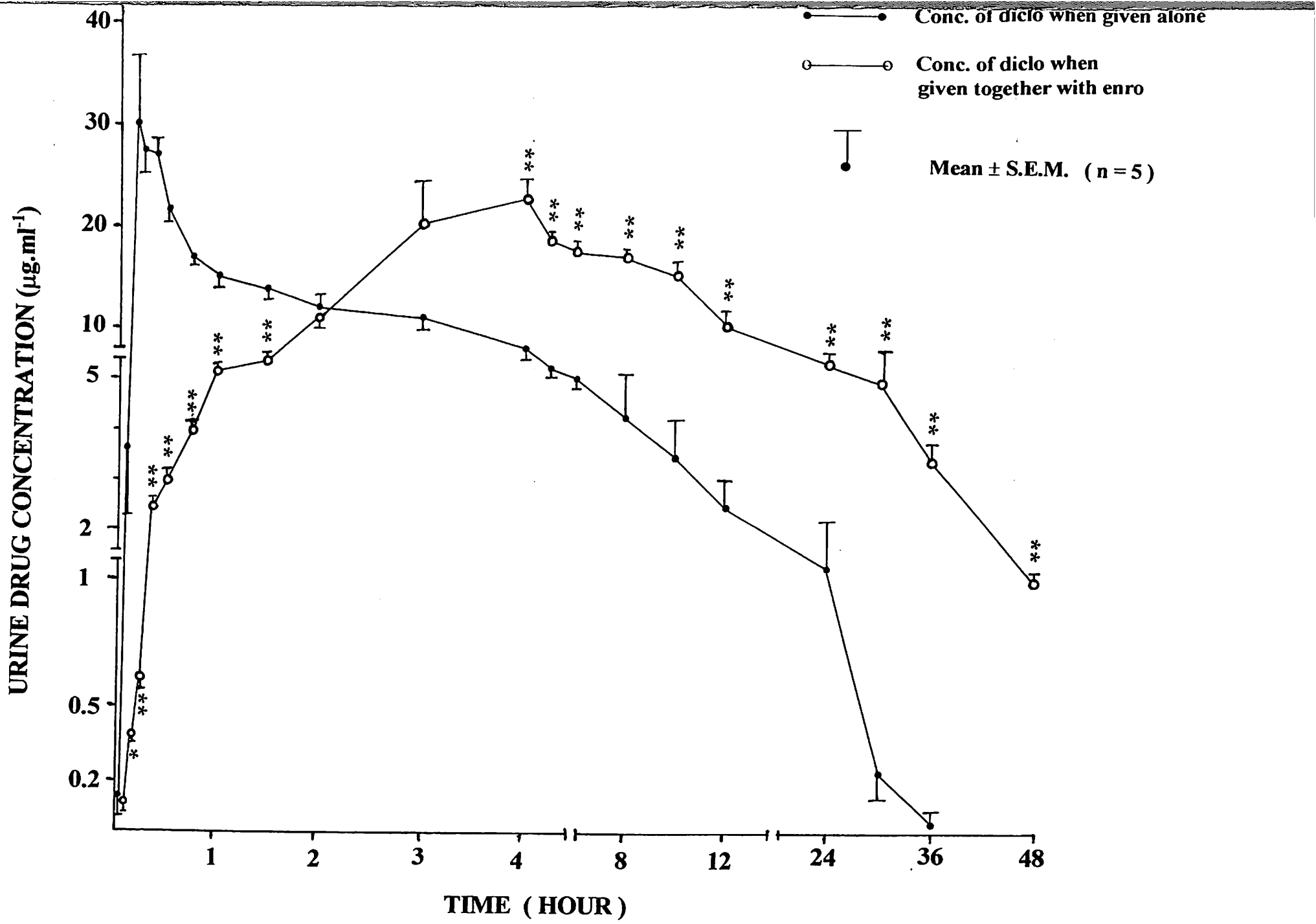
The drug concentrations in urine following single intravenous administration of diclofenac (1 $mg.kg^{-1}$) have been presented in Table 11 and Fig. 5 . The drug appeared at 0.042 h in two out of five animals with a mean value of 0.14 ± 0.09 $kg.ml^{-1}$ while the drug appeared in all five animals at 0.083 h and was maintained upto 24 h in all animals with a mean value of 1.29 ± 0.18 $\mu g.ml^{-1}$. The

Table - 11

Urine concentrations (μg ml^{-1}) of diclofenac in buffalo calves following single intravenous dose of 1 $mg.kg^{-1}$.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	N. D.	N. D.	N. D.	0.45	0.26	0.14 ± 0.09
0.083	0.50	2.52	8.24	4.82	2.28	3.67 ± 1.33
0.167	52.36	25.65	18.96	36.55	16.54	30.01 ± 6.58
0.25	28.33	35.50	23.82	25.16	24.25	27.41 ± 2.17
0.333	25.48	28.60	27.09	22.45	31.62	27.05 ± 1.53
0.50	20.98	22.85	22.73	18.54	24.15	21.85 ± 0.97
0.75	17.03	17.68	18.46	16.28	18.54	17.60 ± 0.43
1	15.49	15.55	17.50	12.45	16.12	15.42 ± 0.83
1.5	13.44	13.86	16.25	11.68	14.15	13.88 ± 0.73
2	10.83	11.22	15.87	10.52	12.88	12.26 ± 0.99
3	9.82	10.15	15.07	9.82	10.45	11.06 ± 1.01
4	5.74	8.82	10.14	7.65	9.12	8.29 ± 0.75
5	5.26	4.56	8.41	5.25	6.62	6.02 ± 0.68
6	4.48	4.10	7.80	4.64	4.00	5.00 ± 0.71
8	3.73	3.80	7.28	3.92	2.80	4.31 ± 0.77
10	3.04	3.10	6.44	2.84	2.10	3.50 ± 0.76
12	2.06	2.16	4.33	2.42	1.58	2.51 ± 0.47
24	1.51	1.22	0.98	1.85	0.88	1.29 ± 0.18
30	0.29	0.38	N. D.	0.52	N. D.	0.24 ± 0.10
36	0.19	N. D.	-	N. D.	-	0.04 ± 0.04
48	N. D.	-	-	-	-	-

N.D. = Non-detectable


Conc. of diclo when given alone
Conc. of diclo when given together with enro
Mean ± S.E.M. (n = 5)
URINE DRUG CONCENTRATION (μg.ml-1)
40
30
20
10
5
2
1
0.5
0.2
1
2
3
4
8
12
24
36
48
TIME (HOUR)

drug appeared in three out of five animals at 30 h with a mean value of 0.24 ± 0.10 $\mu g.ml^{-1}$ and appeared in one animal only at 36 h. The mean peak urine concentration of 30.01 ± 6.58 $\mu g.ml^{-1}$ was observed at 0.167 h.

II. PHARMACOKINETIC STUDY OF DRUGS AFTER COMBINED I.V. ADMINISTRATION OF ENROFLOXACIN AND DICLOFENAC.

[A] Kinetic study of enrofloxacin :

ENROFLOXACIN

1. Plasma levels :

Plasma concentrations of enrofloxacin at various time intervals following combined intravenous administration of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$) have been shown in Table 12 and Fig.1. The drug was present at 0.042 h with a mean of 1.47 ± 0.20 $\mu g.ml^{-1}$ and was detectable in plasma samples of all the buffalo calves up to 8 h with a mean value of 0.08 ± 0.03 $\mu g.ml^{-1}$. The drug was detectable in four out of five animals at 10 & 12 h and none of the animals at 24 h.

2. Kinetic Parameters :

Plasma drug concentration versus time profile has confirmed the two-compartment open model as depicted in Fig.2. Table 13 shows the values of different kinetic parameters calculated by the above noted compartment model.

Table - 12

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin following combined intravenous dose of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$) in buffalo calves.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	0.86	1.76	1.14	1.82	1.75	1.47 ± 0.20
0.083	0.76	1.21	1.00	1.43	1.35	1.15 ± 0.12
0.167	0.62	1.02	0.91	1.18	1.14	0.97 ± 0.10
0.25	0.54	0.85	0.80	1.00	0.99	0.84 ± 0.08
0.333	0.44	0.68	0.66	0.91	0.90	0.72 ± 0.09
0.50	0.39	0.44	0.56	0.75	0.82	0.59 ± 0.08
0.75	0.36	0.40	0.45	0.54	0.66	0.48 ± 0.05
1	0.29	0.23	0.40	0.40	0.50	0.36 ± 0.05
1.5	0.28	0.13	0.34	0.35	0.38	0.30 ± 0.04
2	0.27	0.11	0.27	0.29	0.29	0.25 ± 0.03
3	0.26	0.08	0.21	0.22	0.26	0.21 ± 0.03
4	0.25	0.05	0.16	0.16	0.23	0.17 ± 0.04
5	0.22	0.03	0.13	0.12	0.21	0.14 ± 0.03
6	0.21	0.02	0.10	0.09	0.19	0.12 ± 0.03
8	0.16	0.01	0.06	0.05	0.14	0.08 ± 0.03
10	0.08	N. D.	0.04	0.03	0.09	0.05 ± 0.02
12	0.06	-	0.02	0.01	0.05	0.03 ± 0.01
24	N. D.	-	N. D.	N. D.	N. D.	-

N.D. = Non-detectable

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration (C_p^0=A+B) were noted to be 0.98±0.16, 0.43 ± 0.05 and 1.41 ± 0.17 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 2.26 to 5.60 h^{-1} with a mean value of 3.34 ± 0.60 h^{-1} while its elimination rate constant ranged from 0.14 to 0.43 h^{-1} with a mean value of 0.26 ± 0.05 h^{-1}. The mean distribution half life ($t_{½}\alpha$) and elimination half life ($t_{½}\beta$) of the drug were observed to be 0.23 ± 0.03 and 3.12 ± 0.62 h. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.59 ±0.39 , 1.29 ± 0.28 and 0.72 ± 0.19 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.38 ± 0.03 and 1.70 ± 0.23 . The value of area under curve in plasma (AUC) was found to be 2.28 ± 0.37 $mg.L^{-1}.h$. The various values of volume of distribution calculated by different methods are shown in Table 13. The mean value of Vd_{area} was calculated to be 7.87 ± 0.67 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 20.83 to 61.17 $ml.kg^{-1}.min^{-1}$ with a mean of 33.67 ± 7.22 $ml.kg^{-1}.min^{-1}$.

Table - 13

Kinetic parameters of enrofloxacin calculated by 2 – compartment open model following combined intravenous dose of enrofloxacin (4 mg.kg^{-1}) in buffalo calves.

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A (μg. ml^{-1})	0.57	1.26	0.61	1.24	1.24	0.98 ± 0.16
B (μg. ml^{-1})	0.39	0.28	0.45	0.57	0.46	0.43 ± 0.05
C_p^0 (μg. ml^{-1})	0.96	1.54	1.06	1.81	1.70	1.41 ± 0.17
α (h^{-1})	5.60	2.84	2.26	3.43	2.58	3.34 ± 0.60
$t_{½}$ α (h)	0.12	0.24	0.31	0.20	0.27	0.23 ± 0.03
β (h^{-1})	0.14	0.43	0.25	0.32	0.17	0.26 ± 0.05
$t_{½}$ β (h)	4.95	1.61	2.77	2.17	4.08	3.12 ± 0.62
AUC (mg.L^{-1}.h)	2.89	1.09	2.07	2.14	3.19	2.28 ± 0.37
AUMC (mg. L^{-1}.h^2)	19.92	1.67	7.32	5.67	16.10	10.14 ± 3.40
MRT (h)	6.89	1.53	3.54	2.65	5.05	3.93 ± 0.94
K_{12} (h^{-1})	3.05	1.00	0.90	1.61	1.40	1.59 ± 0.39
K_{21} (h^{-1})	2.36	0.87	1.10	1.30	0.82	1.29 ± 0.28
Kel (h^{-1})	0.33	1.40	0.51	0.84	0.53	0.72 ± 0.19
Fc	0.42	0.31	0.49	0.38	0.32	0.38 ± 0.03
T ≈ P	1.37	2.27	1.06	1.64	2.15	1.70 ± 0.23
Vdc (L.kg^{-1})	4.17	2.60	3.77	2.21	2.35	3.02 ± 0.40
Vd_B (L.kg^{-1})	10.26	14.29	8.89	7.02	8.70	9.83 ± 1.23
Vd_{area} (L.kg^{-1})	9.89	8.53	7.73	5.84	7.38	7.87 ± 0.67
Vd_{ss} (L. kg^{-1})	9.56	5.59	6.85	4.95	6.36	6.66 ± 0.79
Cl_B (ml.kg^{-1}.min^{-1})	23.00	61.17	32.17	31.17	20.83	33.67 ± 7.22

CIPROFLOXACIN

1. Plasma levels :

Plasma concentrations of ciprofloxacin at various time intervals following combined i. v. administration of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$) have been shown in Table 14 and Fig.1. The mean plasma concentration at 0.042 h was noted to be 0.18 ± 0.02 $\mu g.ml^{-1}$. The drug was detectable in plasma samples of four out of five animals at 8 h (0.02 ± 0.01 $\mu g.ml^{-1}$), in three out of five animals at 10 h the mean plasma concentration was noted to be 0.02 ±0.01 $\mu g.ml^{-1}$ and none of the animals at 12 h.

2. Kinetic parameters :

Plasma drug concentration versus time profile of ciprofloxacin had shown non-linear pattern and hence, it can be best described by non-compartmental model. Table 16 shows the values of different kinetic parameters calculated by the non-compartmental analysis.

The elimination rate constant (k or β) ranged from 0.22 to 0.59 h^{-1} with a mean value of 0.41 ± 0.06 h^{-1} while the elimination half life ($t_{½}$ β) ranged from 1.17 to 3.10 h with a mean value of 1.87 ± 0.03 h. The area under curve in plasma (AUC) and area under first moment curve (AUMC) were noted to be 0.82 ± 0.07 $mg.L^{-1}.h$ and 2.22 ± 0.41 $mg.L^{-1}.h^2$ with the mean residential time (MRT) of 2.70 ± 0.48 h . The mean value of volume of distribution at steady state

Table - 14

Plasma concentrations ($\mu g.ml^{-1}$) of ciprofloxacin following combined intravenous dose of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$) in buffalo calves.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	0.13	0.23	0.21	0.20	0.12	0.18 ± 0.02
0.083	0.15	0.27	0.24	0.28	0.20	0.23 ± 0.02
0.167	0.16	0.30	0.29	0.36	0.28	0.28 ± 0.03
0.25	0.19	0.37	0.32	0.50	0.32	0.34 ± 0.05
0.333	0.17	0.35	0.36	0.55	0.38	0.36 ± 0.06
0.50	0.15	0.29	0.33	0.35	0.32	0.29 ± 0.03
0.75	0.13	0.25	0.28	0.28	0.26	0.24 ± 0.03
1	0.12	0.17	0.23	0.24	0.18	0.19 ± 0.02
1.5	0.10	0.16	0.19	0.20	0.12	0.15 ± 0.02
2	0.09	0.14	0.15	0.14	0.07	0.12 ± 0.01
3	0.08	0.09	0.11	0.12	0.06	0.09 ± 0.01
4	0.07	0.07	0.08	0.08	0.03	0.07 ± 0.01
5	0.06	0.04	0.06	0.05	0.02	0.05 ± 0.01
6	0.05	0.03	0.04	0.04	0.01	0.03 ± 0.01
8	0.04	0.02	0.02	0.03	N.D.	0.02 ± 0.01
10	0.03	0.01	N.D.	0.01	-	0.01 ± 0.01
12	N.D.	N.D.	-	N.D.	-	-
24	-	-	-	-	-	-

N.D. = Non-detectable

Table – 15

Kinetic parameters of ciprofloxacin calculated by non-compartmental model following combined intravenous dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}) in buffalo calves.

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
k or β (h^{-1})	0.22	0.38	0.39	0.48	0.59	0.41 ± 0.06
$t_{½}$ β (h)	3.10	1.84	1.80	1.44	1.17	1.87 ± 0.03
AUC (mg. L^{-1}.h)	0.79	0.82	0.93	0.98	0.56	0.82 ± 0.07
AUMC (mg.L^{-1}.h^2)	3.54	2.18	2.41	2.04	0.95	2.22 ± 0.41
MRT (h)	4.48	2.66	2.59	2.08	1.69	2.70 ± 0.48
Vd_{SS} (L.kg^{-1})	23.00	12.84	11.03	8.50	12.10	13.49 ± 2.49
Cl_B (ml.kg^{-1}.min^{-1})	84.40	81.30	71.68	68.00	119.0	84.88 ± 9.05
% conversion of enrofloxacin to ciprofloxacin $\left(\frac{\text{AUC cipro}}{\text{AUC enro}}\right)$	27.34	75.23	44.93	45.79	17.55	42.17 ± 9.85

(Vd_{SS}) was calculated to be 13.49 ± 2.49 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 68.00 to 119.0 with a mean of 84.88 ± 9.05 $ml.kg^{-1}.min^{-1}$. The percentage conversion of enrofloxacin to ciprofloxacin ranged from 17.55 to 75.23 with a mean of 42.17 ± 9.85.

ENROFLOXACIN + CIPROFLOXCIN

1. Plasma Levels :

Plasma concentrations of enrofloxacin + ciprofloxacin together in buffalo calves following combined intravenous administration of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$) are presented in Table 16. The mean plasma concentration of the drug at 0.042 h was found to be 1.65 ± 0.20 $\mu g.ml^{-1}$ and the value ranged from 0.99 to 2.02 $\mu g.\ ml^{-1}$. The drug was detectable in four out of five animals at 12 h and the mean plasma concentration was 0.03 ± 0.01 $\mu g.ml^{-1}$. The drug was not detected at 24 h. The minimum therapeutic concentration (≥ 0.125 $\mu g.\ ml^{-1}$) was maintained up to 6 h.

2. Kinetic parameters :

Plasma drug concentration versus time profile has confirmed the two-compartment open model. Table 17 shows the values of important kinetic parameters of enrofloxacin + ciprofloxacin together needed for calculation of dosage regimen of enrofloxacin in buffalo calves calculated by the above noted compartment model.

Table - 16

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin + ciprofloxacin together in buffalo calves following combined intravenous dose of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	0.99	1.99	1.35	2.02	1.88	1.65 ± 0.20
0.083	0.91	1.48	1.24	1.71	1.55	1.38 ± 0.14
0.167	0.78	1.32	1.20	1.54	1.42	1.25 ± 0.13
0.25	0.73	1.22	1.12	1.50	1.31	1.18 ± 0.13
0.333	0.61	1.03	1.02	1.46	1.28	1.08 ± 0.14
0.50	0.54	0.73	0.89	1.10	1.14	0.88 ± 0.11
0.75	0.49	0.65	0.73	0.82	0.92	0.72 ± 0.07
1	0.41	0.40	0.63	0.64	0.68	0.55 ± 0.06
1.5	0.38	0.29	0.53	0.55	0.50	0.45 ± 0.05
2	0.36	0.25	0.42	0.43	0.36	0.36 ±0.03
3	0.34	0.17	0.32	0.34	0.32	0.30 ± 0.03
4	0.32	0.12	0.24	0.24	0.26	0.24 ± 0.03
5	0.28	0.07	0.19	0.17	0.23	0.19 ± 0.03
6	0.26	0.05	0.14	0.13	0.20	0.16 ± 0.03
8	0.20	0.03	0.08	0.08	0.14	0.11 ± 0.03
10	0.11	0.01	0.04	0.04	0.09	0.06 ± 0.02
12	0.06	N.D.	0.02	0.01	0.05	0.03 ± 0.01
24	N.D.	-	N.D.	N.D.	N.D.	-

N.D. = Non-detectable

Table – 17

Important kinetic parameters of enrofloxacin + ciprofloxacin together needed for calculation of dosage regimen of enrofloxacin calculated by 2-compartment open model following combined i.v. dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}).

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A (μg. ml^{-1})	0.25	1.55	0.66	0.93	1.33	0.94 ± 0.23
B (μg. ml^{-1})	0.58	0.53	0.82	1.00	0.58	0.70 ± 0.09
α (h^{-1})	2.59	3.24	2.84	2.43	1.87	2.59 ± 0.23
$t_{½}$ α (h)	0.27	0.21	0.24	0.29	0.37	0.28 ± 0.03
β (h^{-1})	0.17	0.39	0.30	0.35	0.19	0.28 ± 0.04
$t_{½}$ β (h)	4.19	1.79	2.29	1.98	3.62	2.77 ± 0.48
AUC (mg.L^{-1}.h)	3.51	1.84	2.97	3.24	3.76	3.06 ± 0.33
Vd_{area} (L.kg^{-1})	6.70	5.58	4.50	3.53	5.60	5.18 ± 0.54

The mean extrapolated zero time concentration of enrofloxacin + ciprofloxacin together in plasma during distribution phase (A) and elimination phase (B) were noted to be 0.94 ± 0.23 and 0.70 ± 0.09 μg. ml^{-1}. The distribution rate constant (α) ranged from 1.87 to 3.24 h^{-1} with a mean value of 2.59 ± 0.23 h^{-1} while its elimination rate constant (β) ranged from 0.17 to 0.39 h^{-1} with a mean value of 0.28 ± 0.04 h^{-1}. The mean distribution half life ($t_{½}$ α) and elimination half-life ($t_{½}$ β) were observed to be 0.28 ± 0.03 and 2.77 ± 0.48 h. The value of area under the curve in plasma (AUC) was found to be 3.06 ± 0.33 mg.L^{-1}.h. The mean value of Vd_{area} was calculated to be 5.18 ± 0.54 L.kg^{-1}.

3. Dosage regimen :

The dosage regimen required to maintain the different levels of therapeutic concentration (C_p^{∞}min = 0.125, 0.25 and 0.50 μg.ml^{-1}) in plasma for i.v. route in buffalo calves following combined intravenous dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}) at different dosage intervals (γ) of 8 and 12 h is presented in Table 18. For maintaining C_p^{∞}min of 0.125 μg.ml^{-1}, the loading doses (D*) were calculated to be 7.14 ± 2.31 and 27.69 ± 12.64 mg.kg^{-1} while maintenance doses (D_0) were calculated to be 6.49 ± 2.32 and 27.04 ± 12.65 mg. kg^{-1} at the dosage intervals (γ) of 8 and 12 h, respectively. The D*s were calculated to be 14.28 ± 4.61 and 55.38 ± 25.27 mg.kg^{-1} while D_0s were found to be 12.99 ± 4.63 and 54.08 ± 25.29 mg.kg^{-1} at

Table - 18

Dosage regimen of enrofloxacin for intravenous route in buffalo calves following combined i.v. dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}).

C_p^{α} min ($\mu g.\ ml^{-1}$)	γ(h)	Dose ($mg.kg^{-1}$)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
0.125	8	D*	3.26	15.79	6.20	7.25	3.20	7.14 ± 2.31
		D_0	2.43	15.09	5.64	6.81	2.50	6.49 ± 2.32
	12	D*	6.44	75.17	20.59	29.42	6.84	27.69 ± 12.64
		D_0	5.60	74.47	20.02	28.98	6.14	27.04 ± 12.65
0.25	8	D*	6.52	31.58	12.40	14.50	6.40	14.28 ± 4.61
		D_0	4.86	30.18	11.28	13.62	5.00	12.99 ± 4.63
	12	D*	12.88	150.3	41.18	58.84	13.68	55.38 ± 25.27
		D_0	11.20	148.9	40.04	57.96	12.28	54.08 ± 25.29
0.50	8	D*	13.45	63.16	24.80	29.00	12.80	28.56 ± 9.22
		D_0	9.72	60.36	22.56	27.24	10.00	25.98 ± 9.26
	12	D*	25.76	300.7	82.36	117.7	27.36	110.8 ± 50.55
		D_0	22.40	297.9	80.08	115.9	24.56	108.2 ± 50.59

D* = Priming or Loading dose

D_0 = Maintenance dose

γ = Dosage interval

C_p^{α} min = Minimum therapeutic concentration in plasma (MIC)

γ of 8 and 12 h, for maintaining C_p^{∞} min of 0.25 μg.ml^{-1}. Similarly, to maintain C_p^{∞} min of 0.50 μg.ml^{-1} the D*s were calculated to be 28.56 ± 9.22 and 110.8 ± 50.55 mg.kg^{-1} while D_0s were found to be 25.98 ± 9.26 and 108.2 ± 50.59 mg.kg^{-1} at γ of 8 and 12 h.

4. Urine levels :

The concentration of enrofloxacin (including its active metabolite ciprofloxacin) in urine estimated by microbiological assay in buffalo calves following combined intravenous dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}) have been depicted in Table 19 and Fig. 3. The drug appeared in effective therapeutic concentration (0.125 μg.ml^{-1}) in all five animals at 0.042 h and was maintained even beyond 48 h. The mean peak urine concentration of 160.2 ± 11.92 μg.ml^{-1} was observed at 0.333 h. The drug was detectable in all five animals at 48 h (0.24 ± 0.04 μg.ml^{-1}).

[B] <u>Kinetic Study of diclofenac</u> :

The kinetic study of diclofenac in buffalo calves following combined intravenous dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}) in plasma and urine simple was estimated by HPLC method.

1. Plasma levels :

Plasma concentrations of diclofenac at various time intervals following combined i.v. dose of enrofloxacin (4 mg.kg^{-1}) and

Table - 19

Urine concentrations ($\mu g.ml^{-1}$) of enrofloxacin (including its active metabolite ciprofloxacin) estimated by microbiological assay in buffalo calves following combined intravenous dose of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	3.03	3.95	4.01	3.10	3.35	3.49 ± 0.21
0.083	22.22	25.43	42.31	24.52	25.01	27.90 ± 3.65
0.167	55.72	65.23	92.45	56.21	55.97	65.12 ± 7.07
0.25	105.6	101.4	119.9	106.8	106.9	108.1 ± 3.11
0.333	183.8	121.9	145.6	165.2	184.3	160.2 ± 11.92
0.50	121.3	140.6	184.9	181.9	123.7	150.5 ± 13.85
0.75	105.6	185.2	165.3	125.2	107.9	137.8 ± 15.96
1	91.90	160.1	135.4	104.9	94.35	117.3 ± 13.20
1.5	69.64	142.3	109.6	93.34	71.27	97.23 ± 13.48
2	62.34	108.7	92.31	71.24	64.35	79.79 ± 8.97
3	40.00	85.92	82.38	46.41	46.92	60.33 ± 9.82
4	32.04	65.75	63.24	37.40	37.62	47.21 ± 7.14
5	24.28	35.42	33.31	25.31	28.42	29.35 ± 2.18
6	17.41	25.91	26.01	18.61	20.29	21.65 ± 1.82
8	14.74	18.26	19.31	15.12	16.32	16.75 ± 0.89
10	8.34	10.17	11.21	9.31	10.35	9.88 ± 0.49
12	6.92	7.38	8.02	6.52	7.21	7.21 ± 0.25
24	4.01	6.21	5.31	3.93	5.03	4.90 ± 0.43
30	2.06	3.40	2.62	1.96	2.16	2.44 ± 0.27
36	0.60	0.90	0.96	0.72	0.95	0.83 ± 0.07
48	0.16	0.39	0.23	0.19	0.21	0.24 ± 0.04

diclofenac (1 $mg.kg^{-1}$) in buffalo calves have been shown in Table 20 and Fig 4. The mean drug concentration in plasma at 0.042 h was found to be 2.45 ± 0.15 $\mu g.ml^{-1}$ and the value ranged from 1.96 to 2.76 $\mu g.ml^{-1}$. The drug was detectable in all five animals at 24 h and the mean plasma drug concentration was noted to be 0.19 ± 0.02 $\mu g.ml^{-1}$.

2. *Kinetic parameters :*

Plasma drug concentration versus time profile has confirmed the two-compartment open model. Table 21 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0=A+B) were noted to be 1.21 ±0.22, 0.73 ± 0.03 and 1.94 ± 0.22 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.96 to 7.45 h^{-1} with a mean value of 4.47 ± 1.09 h^{-1} while its elimination rate constant (β) ranged from 0.04 to 0.07 h^{-1} with a mean value of 0.06 ± 0.01 h^{-1}. The mean distribution half life ($t_{½}\ \alpha$) and elimination half life ($t_{½}\ \beta$) values of the drug were observed to be 0.21 ± 0.06 and 12.84 ± 1.29 h. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 2.64 ± 0.79, 1.75 ± 0.41 and 0.14 ± 0.01 h^{-1}, respectively. The fraction of drug available for

Table - 20

Plasma concentrations ($\mu g.ml^{-1}$) of diclofenac in buffalo calves following combined intravenous dose of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	2.63	1.96	2.69	2.22	2.76	2.45 ± 0.15
0.083	1.83	1.25	1.68	1.34	1.70	1.56 ± 0.11
0.167	1.24	1.08	1.30	1.12	1.35	1.22 ± 0.05
0.25	1.02	0.96	1.08	0.98	1.09	1.03 ± 0.03
0.333	0.96	0.91	0.98	0.94	0.99	0.96 ± 0.01
0.50	0.75	0.82	0.94	0.85	0.95	0.86 ± 0.04
0.75	0.73	0.78	0.88	0.80	0.88	0.81 ± 0.03
1	0.70	0.74	0.80	0.77	0.81	0.76 ± 0.02
1.5	0.68	0.71	0.68	0.72	0.70	0.70 ± 0.01
2	0.67	0.68	0.66	0.70	0.68	0.68 ± 0.01
3	0.63	0.65	0.59	0.68	0.62	0.63 ± 0.02
4	0.60	0.62	0.50	0.65	0.55	0.58 ± 0.03
5	0.58	0.60	0.43	0.62	0.48	0.54 ± 0.04
6	0.57	0.59	0.39	0.58	0.42	0.51 ± 0.04
8	0.56	0.55	0.31	0.55	0.35	0.46 ± 0.06
10	0.50	0.48	0.24	0.50	0.28	0.40 ± 0.06
12	0.45	0.44	0.20	0.46	0.24	0.36 ± 0.06
24	0.24	0.19	0.14	0.22	0.18	0.19 ± 0.02

Table - 21

Kinetic parameters of diclofenac in buffalo calves calculated by 2- compartment open model following combined intravenous dose of enrofloxacin (4 mg.kg^{-1}) and diclofenac (1 mg.kg^{-1}).

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A (μg. ml^{-1})	2.06	0.96	1.07	0.85	1.09	1.21 ± 0.22
B (μg. ml^{-1})	0.74	0.80	0.65	0.80	0.67	0.73 ± 0.03
C_p^0 (μg. ml^{-1})	2.80	1.76	1.72	1.65	1.76	1.94 ± 0.22
α (h^{-1})	7.45	6.23	1.96	4.61	2.11	4.47 ± 1.09
$t_{½}$ α (h)	0.09	0.11	0.35	0.15	0.33	0.21 ± 0.06
β (h^{-1})	0.04	0.06	0.07	0.05	0.06	0.06 ± 0.01
$t_{½}$ β (h)	17.33	11.55	9.90	13.86	11.55	12.84 ± 1.29
AUC (mg.L^{-1}.h)	18.78	13.49	9.83	16.18	11.68	13.99 ± 1.59
AUMC (mg. L^{-1}.h^2)	462.5	222.3	132.9	320.0	186.4	264.8 ± 58.10
MRT (h)	24.63	16.48	13.52	19.78	15.96	18.07 ± 1.92
K_{12} (h^{-1})	5.34	3.30	1.07	2.30	1.18	2.64 ± 0.79
K_{21} (h^{-1})	2.00	2.86	0.78	2.26	0.84	1.75 ± 0.41
Kel (h^{-1})	0.15	0.13	0.18	0.10	0.15	0.14 ± 0.01
Fc	0.27	0.46	0.39	0.50	0.40	0.40 ± 0.04
T ≈ P	2.72	1.18	1.51	1.04	1.51	1.59 ± 0.30
Vdc (L.kg^{-1})	0.36	0.57	0.58	0.61	0.57	0.54 ± 0.05
Vd_B (L.kg^{-1})	1.35	1.25	1.54	1.25	1.49	1.38 ± 0.06
Vd_{area} (L.kg^{-1})	1.33	1.24	1.45	1.24	1.43	1.34 ± 0.04
Vd_{SS} (L.kg^{-1})	1.32	1.23	1.38	1.23	1.37	1.31 ± 0.03
Cl_B (ml.kg^{-1}.min^{-1})	0.84	1.17	1.67	1.00	1.50	1.24 ± 0.15

elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.40 ± 0.04 and 1.59 ± 0.30, respectively. The value of area under curve in plasma (AUC) and area under first moment curve (AUMC) were found to be 13.99 ± 1.59 $mg.L^{-1}.h$ and 264.8 ± 58.10 $mg.L^{-1}.h^2$ with the mean residential time (MRT) of 18.07 ± 1.92 h. The various values of volume of distribution calculated by different methods are shown in Table 21. The mean value of Vd_{area} was calculated to be 1.34 ± 0.04 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 0.84 to 1.67 $ml.kg^{-1}.min^{-1}$ with a mean of 1.24 ± 0.15 ml. $kg^{-1}.min^{-1}$.

3. Urine levels :

Urine concentrations of diclofenac in buffalo calves following combined intravenous dose of enrofloxacin (4 mg. kg^{-1}) and diclofenac (1 mg. kg^{-1}) are presented in Table 22 and Fig. 5. The drug was not at all detected in any of the five animals at 0.042 h. The drug appeared in four out of five animals with a mean value of 0.12 ± 0.05 $\mu g.ml^{-1}$ at 0.083 h. The mean peak urine concentration of 22.80 ± 1.34 $\mu g.ml^{-1}$ was observed at 4 h. The drug was present in all five animals up to 48 h with a mean value of 1.03 ± 0.07 $\mu g.ml^{-1}$.

Table - 22

Urine concentrations ($\mu g.ml^{-1}$) of diclofenac in buffalo calves following combined intravenous dose of enrofloxacin (4 $mg.kg^{-1}$) and diclofenac (1 $mg.kg^{-1}$).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	N.D.	N.D.	N.D.	N.D.	N.D.	-
0.083	-	0.22	-	0.20	0.18	0.12 ± 0.05
0.167	0.34	0.40	0.38	0.36	0.42	0.38 ± 0.01
0.25	0.53	0.68	0.56	0.60	0.75	0.62 ± 0.04
0.333	2.37	2.48	2.40	2.36	2.86	2.49 ± 0.09
0.50	2.74	3.12	2.92	2.95	3.45	3.04 ± 0.12
0.75	3.98	4.15	3.90	4.00	4.28	4.06 ± 0.07
1	5.36	6.12	6.10	5.85	6.16	5.92 ± 0.15
1.5	6.94	7.08	6.55	6.62	7.12	6.86 ± 0.12
2	10.01	12.16	10.44	11.78	10.86	11.05 ± 0.40
3	11.73	28.50	12.43	26.60	22.65	20.38 ± 3.52
4	23.72	20.15	25.50	19.12	25.52	22.80 ± 1.34
5	18.62	18.85	19.12	17.88	19.00	18.69 ± 0.22
6	17.30	18.00	18.68	17.00	18.25	17.85 ± 0.31
8	16.50	17.16	17.28	16.82	17.00	16.95 ± 0.14
10	15.52	16.18	16.10	11.45	15.94	15.04 ± 0.90
12	14.45	9.45	10.00	7.82	9.82	10.31 ± 1.10
24	8.31	6.50	7.15	5.12	7.05	6.83 ± 0.52
30	7.37	4.22	4.88	3.92	4.46	4.97 ± 0.62
36	4.48	3.15	3.25	3.10	3.20	3.44 ± 0.26
48	0.90	1.10	1.22	0.85	1.06	1.03 ± 0.07

N.D. = Non-detectable.

III. COMPARISON OF PHARMACOKINETICS OF ENROFLOXACIN AND CIPROFLOXACIN WHEN ENROFLOXACIN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH DICLOFENAC BY I. V. ADMINISTRATION :

ENROFLOXACIN

1. Plasma levels :

Comparative plasma concentrations of enrofloxacin administered alone and in combination with diclofenac after its i. v. administration are shown in Table 23 and Fig.1. The drug was present in plasma upto 12 h in both the groups. The minimum therapeutic concentration ($\geq 0.125\ \mu g.ml^{-1}$) of enrofloxacin (including its active metabolite ciprofloxacin) was maintained upto 6 h in both the groups. All data are noted to be non-significant from 0.042 h to 12 h on statistical comparison between the two groups.

2. Kinetic parameters :

Table 24 reveals the comparison of kinetic parameters of enrofloxacin when administered alone and in combination with diclofenac after i.v. administration. No significant difference was obtained in any of the kinetic parameters when enrofloxacin given alone and when enrofloxacin given together with diclofenac.

Table - 23

Comparison of plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin and ciprofloxacin when enrofloxacin (4 $mg.kg^{-1}$) given alone and when given together with diclofenac (1 $mg.kg^{-1}$) in buffalo calves following intravenous administration.

Time (h)	Enrofloxacin given alone		Enrofloxacin + diclofenac combined administration	
	Enrofloxacin	Ciprofloxacin	Enrofloxacin	Ciprofloxacin
0.042	2.61 ± 1.12	0.07 ± 0.04	1.47± 0.20	0.08 ±0.02
0.083	2.05 ± 0.82	0.16 ± 0.06	1.15 ±0.12	0.23 ±0.02
0.167	1.56 ± 0.54	0.25 ± 0.04	0.97 ±0.10	0.28 ±0.03
0.25	1.20 ± 0.30	0.38 ± 0.14	0.84 ±0.08	0.34 ±0.05
0.333	0.97 ± 0.23	0.32 ± 0.04	0.72 ±0.09	0.36 ±0.06
0.50	0.72 ± 0.13	0.32 ± 0.03	0.59 ±0.08	0.29 ±0.03
0.75	0.55 ± 0.10	0.26 ± 0.03	0.48 ±0.05	0.24 ±0.03
1	0.46 ± 0.07	0.23 ± 0.03	0.36 ±0.05	0.19 ±0.02
1.5	0.32 ± 0.06	0.18 ± 0.02	0.30 ±0.04	0.15 ±0.02
2	0.27 ± 0.05	0.16 ± 0.02	0.25 ±0.03	0.12 ±0.01
3	0.20 ± 0.03	0.11 ± 0.02	0.21 ±0.03	0.09 ±0.01
4	0.16 ± 0.02	0.08 ± 0.02	0.17 ±0.04	0.07 ±0.01
5	0.13 ± 0.02	0.07 ± 0.02	0.14 ±0.03	0.05 ±0.01
6	0.08 ± 0.02	0.06 ± 0.02	0.12 ±0.03	0.03 ±0.01
8	0.05 ± 0.01	0.04 ± 0.01	0.08 ±0.03	0.02 ±0.01
10	0.04 ± 0.01	0.03 ± 0.01	0.05 ±0.02	0.01 ±0.01
12	0.02 ± 0.01	0.02 ± 0.01	0.03 ±0.01	-
24	-	-	-	-

All data are non-significant.

Table - 24

Comparison of kinetic parameters of enrofloxacin and ciprofloxacin when enrofloxacin (4 mg.kg^{-1}) given alone and when given together with diclofenac (1 mg.kg^{-1}) in buffalo calves following intravenous administration

Parameter (Unit)	Enrofloxacin given alone		Enrofloxacin + diclofenac combined administration	
	Enrofloxacin	Ciprofloxacin	Enrofloxacin	Ciprofloxacin
A (μg. ml^{-1})	1.56 ±0.58		0.98 ±0.16	
B (μg. ml^{-1})	0.41 ±0.06		0.43 ±0.05	
C_p^0 (μg. ml^{-1})	1.97 ±0.55		1.41 ±0.17	
α (h^{-1})	2.65 ±0.41		3.34 ±0.60	
$t_{½}$ α (h)	0.28 ±0.04		0.23 ±0.03	
β (h^{-1})	0.25 ±0.03	0.31 ±0.05	0.26 ±0.05	0.41 ±0.06
$t_{½}$ β (h)	2.92 ±0.41	2.40 ±0.33	3.12 ±0.62	1.87 ±0.03
AUC (mg.L^{-1}.h)	2.37 ±0.45	1.10 ±0.27	2.28 ±0.37	0.82 ±0.07
AUMC (mg. L^{-1}.h^2)	7.44 ±1.67	4.29 ±1.56	10.14± 3.40	2.22 ±0.41
MRT (h)	3.05 ±0.20	3.47 ±0.47	3.93 ±0.94	2.70 ±0.48
K_{12} (h^{-1})	1.17 ±0.27		1.59 ±0.39	
K_{21} (h^{-1})	1.06 ±0.09		1.29 ±0.28	
Kel (h^{-1})	0.68 ±0.15		0.72 ±0.19	
Fc	0.43 ±0.06		0.38 ±0.03	
T ≈ P	1.53 ±0.35		1.70 ±0.23	
Vdc (L.kg^{-1})	2.44 ±0.38		3.02 ±0.40	
Vd_B (L.kg^{-1})	10.63 ±1.40		9.83 ±1.23	
Vd_{area} (L.kg^{-1})	7.47 ±0.69		7.87 ±0.67	
Vd_{SS} (L.kg^{-1})	5.33 ±1.04	13.60 ±1.23	6.66 ±0.79	13.49 ±2.49
Cl_B (ml.kg^{-1}.min^{-1})	32.40 ±5.69	72.38 ±13.17	33.67 ±7.22	84.88 ±9.05
% conversion of enrofloxacin to ciprofloxacin (AUC_{cipro}/AUC_{Enro})		46.96 ± 5.60		42.17 ±9.85

All data are non-significant.

CIPROFLOXACIN

1. Plasma levels :

Comparative plasma concentrations of ciprofloxacin (active metabolite of enrofloxacin) when enrofloxacin given alone and when enrofloxacin given in combination with diclofenac following i. v. administration are shown in Table 23 and Fig. 1. Ciprofloxacin was present in plasma upto 12 h when enrofloxacin given alone while it was present in plasma upto 10 h in case of combined administration of enrofloxacin and diclofenac. All data obtained for ciprofloxacin concentrations in plasma at various time intervals are non-significantly differed between both the groups when enrofloxacin given alone and when given together with diclofenac.

2. Kinetic parameters :

Table 24 shows the comparison of kinetic parameters of ciprofloxacin (active metabolite of enrofloxacin) when enrofloxacin given alone and when given together with diclofenac in buffalo calves following intravenous administration. All kinetic parameters were noted to differ only non-significantly between both the groups.

3. Urine levels :

Table 25 and Fig.3 present the comparison of urine concentrations of enrofloxacin (including its active metabolite ciprofloxacin) estimated by microbiological assay when enrofloxacin given alone and when given together with diclofenac in buffalo calves

Table - 25

Comparison of urine concentration ($\mu g.ml^{-1}$) of enrofloxacin (including its active metabolite ciprofloxacin) estimated by microbiological assay when enrofloxacin ($4mg.kg^{-1}$) given alone and when given together with diclofenac ($1mg.kg^{-1}$) in buffalo calves following i.v. administration.

Time (h)	Enrofloxacin given alone	Enrofloxacin + diclofenac combined administration
0.042	2.90 ± 0.10	3.49 ±0.21
0.083	24.94 ±1.42	27.90 ±3.65
0.167	56.70 ±1.04	65.12 ±7.07
0.25	107.3 ±1.63	108.1 ±3.11
0.333	158.1 ±12.55	160.2 ±11.92
0.50	161.6 ±12.20	150.5 ±13.85
0.75	137.7 ±15.40	137.8 ±15.96
1	106.8 ±6.14	117.3 ±13.20
1.5	86.62 ±6.96	97.23 ±13.48
2	76.86 ±5.08	79.79 ±8.97
3	54.03 ±4.92	60.33 ±9.82
4	42.53 ±4.96	47.21 ±7.14
5	32.80 ±3.53	29.35 ±2.18
6	22.98 ±2.69	21.65 ±1.82
8	18.74 ±1.54	16.75 ±0.89
10	11.75 ± 1.54	9.88 ±0.49
12	7.59 ±0.53	7.21 ±0.25
24	4.68 ±0.42	4.90 ±0.43
30	2.28 ±0.24	2.44 ±0.27
36	0.89 ±0.03	0.83 ±0.07
48	0.23 ±0.03	0.24 ±0.04

All data are non-significant.

Table – 26

Comparison of calculated dosage regimen of enrofloxacin when given alone and when given together with diclofenac in buffalo calves following intravenous administration.

C_p^{∞} min ($\mu g.\ ml^{-1}$)	γ(h)	Dose ($mg.kg^{-1}$)	Enrofloxacin given alone	Enrofloxacin + diclofenac given together
0.125	8	D*	6.07 ± 1.67	7.14 ± 2.31
		D_0	5.45 ± 1.65	6.49 ± 2.32
	12	D*	20.72 ± 7.46	27.69 ± 12.64
		D_0	20.10 ± 7.44	27.04 ± 12.65
0.25	8	D*	12.15 ± 3.35	14.28 ± 4.61
		D_0	10.90 ± 3.30	12.99 ± 4.63
	12	D*	41.44 ± 14.92	55.38 ± 25.27
		D_0	40.20 ± 14.88	54.08 ± 25.29
0.50	8	D*	24.30 ± 6.70	28.56 ± 9.22
		D_0	21.80 ± 6.60	25.98 ± 9.26
	12	D*	82.88 ± 29.84	110.8 ± 50.55
		D_0	80.40 ± 29.76	108.2 ± 50.59

All data are non-significant.

D* = Priming or Loading dose.

D_0 = Maintenance dose

γ = Dosage interval

C_p^{∞} min = Minimum therapeutic concentration in plasma (MIC).

following i. v. administration. The drug was present in urine from 0.042 to 48 h in both the groups. The minimum therapeutic concentration (≥ 0.125 $\mu g.ml^{-1}$) was maintained from 0.042 h to 48 h in both the groups. The peak urine concentrations of 161.6 ± 12.20 and 160.2 ± 11.92 $\mu g.ml^{-1}$ were noted when enrofloxacin given alone and when given together with diclofenac at 0.50 h and 0.333 h, respectively. All data are found to differ non-significantly at various time intervals between both the groups.

4. Dosage Regimen :

Comparison of dosage regimen of enrofloxacin when given alone and when given together with diclofenac for different therapeutic levels (C_p^{∞}min = 0.125, 0.25 and 0.50 $\mu g.ml^{-1}$) and different dosage intervals (γ) of 8 and 12 h have been shown in Table 26. All calculated data for loading (D^*) and maintenance (D_0) doses for different therapeutic levels and different dosage intervals were noted to be non- significant between both the groups.

IV. COMPARISON OF PHARMACOKINETICS OF DICLOFENAC ADMINISTERED ALONE AND IN COMBINATION WITH ENROFLOXACIN BY I.V. ROUTE.

1. Plasma levels :

Comparative plasma concentrations of diclofenac when diclofenac given alone and in combination with enrofloxacin after its i.v. administration are shown in Table 27 and Fig.4. Plasma

Table - 27

Comparison of plasma and urine concentration ($\mu g.ml^{-1}$) of diclofenac ($1\ mg.kg^{-1}$) when given alone and when given together with enrofloxacin ($4\ mg.kg^{-1}$) in buffalo calves following intravenous administrations.

Time (h) ↓	Diclofenac given alone		Enrofloxacin + diclofenac given together	
	Plasma	Urine	Plasma	Urine
0.042	7.04 ± 0.75	0.14 ± 0.09	2.45 ± 0.15**	0.00 ± 0.00+
0.083	5.69 ± 0.68	3.67 ± 1.33	1.56 ± 0.11**	0.12 ± 0.05+
0.167	5.00 ± 0.85	30.01 ± 6.58	1.22 ± 0.05*	0.38 ± 0.01*
0.25	4.33 ± 0.67	27.41 ± 2.17	1.03 ± 0.03**	0.62 ± 0.04**
0.333	3.87 ± 0.59	27.05 ± 1.53	0.96 ± 0.01**	2.49 ± 0.09**
0.50	3.27 ± 0.46	21.85 ± 0.97	0.86 ± 0.04**	3.04 ± 0.12**
0.75	2.42 ± 0.26	17.60 ± 0.43	0.81 ± 0.03**	4.06 ± 0.07**
1	1.93 ± 0.24	15.42 ± 0.83	0.76 ± 0.02**	5.92 ± 0.15**
1.5	1.51 ± 0.16	13.88 ± 0.73	0.70 ± 0.01**	6.86 ± 0.12**
2	1.32 ± 0.12	12.26 ± 0.99	0.68 ± 0.01**	11.05 ± 0.40+
3	0.98 ± 0.10	11.06 ± 1.01	0.63 ± 0.02*	20.38 ± 3.52+
4	0.79 ± 0.08	8.29 ± 0.75	0.58 ± 0.03+	22.80±1.34**
5	0.63 ± 0.04	6.02 ± 0.68	0.54 ± 0.04+	18.69 ± 0.22**
6	0.52 ± 0.04	5.00 ± 0.71	0.51 ± 0.04+	17.85 ± 0.31**
8	0.32 ± 0.05	4.31 ± 0.77	0.46 ± 0.06*	16.95 ± 0.14**
10	0.19 ± 0.03	3.50 ± 0.76	0.40 ± 0.06*	15.04 ± 0.90**
12	0.14 ± 0.02	2.51 ± 0.47	0.36 ± 0.06*	10.31 ± 1.10**
24	0.03 ± 0.01	1.29 ± 0.18	0.19 ± 0.02**	6.83 ± 0.52**
30	-	0.24 ± 0.10	-	4.97 ± 0.62**
36	-	0.04 ± 0.04	-	3.44 ± 0.26**
48	-	0.00 ± 0.00	-	1.03 ± 0.07**

\+ Non-significant * $p < 0.05$ ** $p < 0.01$

concentrations of the drug were found to be significantly lower initially (0.042 to 3 h) and higher later (8 to 24 h) in case of combined administration as compared to single administration of diclofenac. Between 4 to 6 h, the drug concentrations were found to be non-significant between both the groups.

2. *Urine levels :*

The drug appeared at 0.042 h and was detectable upto 36 h in urine when diclofenac was given alone while in combination with enrofloxacin the drug appeared at 0.083 h and was detectable upto 48 h in urine. The study revealed that significantly lower concentrations of diclofenac in urine were found initially from 0.167 to 1.5 h and higher drug concentrations were found later from 4 to 48 h in case of combined administration as compared to single administration of diclofenac. The peak urine drug concentration of 30.01 ± 6.58 $\mu g.ml^{-1}$ was attained earlier at 0.167 h when diclofenac was administered alone while peak urine drug concentration of 22.80 ± 1.34 $\mu g.ml^{-1}$ was achieved later at 4 h in combined administration of diclofenac with enrofloxacin (Table 27 and Fig. 5).

3. *Kinetic parameters :*

The statistical comparison of different kinetic parameters of diclofenac when given alone and when given together with enrofloxacin in buffalo calves following i. v. administration has been depicted in Table 28. The value for extrapolated zero time

Table – 28

Comparison of kinetic parameters of diclofenac when given alone (1 mg.kg^{-1}) and when given together with enrofloxacin (4 mg.kg^{-1}) in buffalo calves following intravenous administration.

Parameter (Unit)	Diclofenac given alone	Enrofloxacin + diclofenac combined administration
A (μg. ml^{-1})	5.74 ± 1.20	1.21 ± 0.22*
B (μg. ml^{-1})	1.65 ± 0.35	0.73 ± 0.03^{+}
C_p^0 (μg. ml^{-1})	7.38 ± 1.49	1.94 ± 0.22*
α (h^{-1})	2.76 ± 0.81	4.47 ± 1.09^{+}
$t_{½}$ α (h)	0.34 ± 0.08	0.21 ± 0.06^{+}
β (h^{-1})	0.19 ± 0.03	0.06 ± 0.01**
$t_{½}$ β (h)	4.06 ± 0.59	12.84 ± 1.29**
AUC (mg.L^{-1}.h)	11.24 ± 0.48	13.99 ± 1.59^{+}
AUMC (mg. L^{-1}.h^2)	51.78 ± 7.30	264.8 ± 58.10*
MRT (h)	4.72 ± 0.85	18.07 ± 1.92**
K_{12} (h^{-1})	1.48 ± 0.53	2.64 ± 0.79^{+}
K_{21} (h^{-1})	0.83 ± 0.22	1.75 ± 0.41^{+}
Kel (h^{-1})	0.64 ± 0.12	0.14 ± 0.01*
Fc	0.30 ± 0.03	0.40 ± 0.04^{+}
T ≈ P	2.43 ± 0.32	1.59 ± 0.30^{+}
Vdc (L.kg^{-1})	0.17 ± 0.05	0.54 ± 0.05*
Vd_B (L.kg^{-1})	0.72 ± 0.13	1.38 ± 0.06*
Vd_{area} (L.kg^{-1})	0.54 ± 0.10	1.34 ± 0.04**
Vd_{SS} (L. kg^{-1})	0.43 ± 0.10	1.31 ± 0.03**
Cl_B (ml.kg^{-1}.min^{-1})	1.52 ± 0.07	1.24 ± 0.15^{+}

$^{+}$ Non-significant * $p < 0.05$ ** $p < 0.01$

concentration in distribution phase (A) and the theoretical zero time concentration (C_p^0) were significantly lower ($p< 0.05$) in combined administration as compared to single administration of diclofenac while the value for extrapolated zero time concentration in elimination phase (B) was found to be non-significant between both the groups. Distribution rate constant (α) and distribution half life ($t_{1/2}\ \alpha$) differed non significantly which denote that the drug may be distributed at a similar rate in both the groups. Highly significant ($P < 0.01$) lower value of $0.06 \pm 0.01\ h^{-1}$ for elimination rate constant (β) and higher value of 12.84 ± 1.29 h for elimination half life ($t_{½}\ \beta$) in combined administration as compared to the value of β ($0.19 \pm 0.03\ h^{-1}$) and $t_{½}\ \beta$ (4.06 ± 0.59 h) when given alone indicate slow elimination of the drug from the body of buffalo calves when both the drugs were administered together. This is further supported by significantly ($p < 0.05$) lower value of rate of elimination of drug from central compartment (Kel) obtained in combined administration. Non-significant variation was observed in the value of rate constant of drug transfer from central to peripheral compartment (K_{12}) and peripheral to central compartment (K_{21}) in both the groups. The area under curve (AUC) was found to be non- significant while area under first moment curve (AUMC) and the mean residential time (MRT) were observed to be highly significant in combined administration as compared to single administration of diclofenac. The fraction of drug

available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were found to be non-significant in both the groups. Various values of volume of distribution were found to be significantly higher when diclofenac given together with enrofloxacin as compared to when diclofenac was given alone in buffalo calves following intravenous administration. The value of total body clearance (Cl_B) was noted to be non-significant between both the groups.

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Chapter – V

Discussion

DISCUSSION

Enrofloxacin, a recent member of fluoroquinolones, possesses many advantages such as bactericidal and broad spectrum activity, no cross resistance with other groups of chemotherapeutic agents and better distribution in different organs and tissues in various species of animals. The drug has been developed exclusively for veterinary use. Though pharmacokinetic studies of enrofloxacin were carried out in many species but little work has been done so far in buffalo calf. Diclofenac, a potent NSAID having analgesic and antipyretic properties is frequently employed in treating inflammatory conditions associated with pyrexia in animals. It seems that kinetic study of diclofenac has not been carried out so far in buffalo calf. Antimicrobial agents are concurrently used along with diclofenac for treating microbial infections as well as to treat inflammatory and febrile conditions. Though pharmacokinetic interactions between antimicrobials and NSAIDs were studied in animals, available literature showed little studies on interaction between enrofloxacin and diclofenac in animals, particularly in buffalo calves. Therefore, the present study was undertaken to know the kinetic interactions of enrofloxacin with diclofenac in buffalo calves.

Kinetic study of enrofloxacin and its active metabolite ciprofloxacin :

(a) Distribution in plasma :

Concentrations of enrofloxacin at various time intervals post i. v. injections of enrofloxacin (4 $mg.kg^{-1}$) when given alone and when given together with diclofenac (1 $mg.kg^{-1}$ i. v.) did not differ significantly (Table 23 and Fig.1). Similarly, concentrations of ciprofloxacin, the active metabolite of enrofloxacin also did not differ significantly at different time intervals. This fact denotes that diclofenac may not have much influence in altering plasma levels of enrofloxacin as well as in influencing the metabolic conversion of enrofloxacin to ciprofloxacin particularly in buffalo calves. In contrast to the present study, Varma *et al.* (2000) noted lower maintenance of therapeutic concentrations of enrofloxacin when given alone as compared to combined administration of enrofloxacin with diclofenac in cattle. Further, they observed that conversion of enrofloxacin to ciprofloxacin was reduced leading to lower plasma concentrations of ciprofloxacin when enrofloxacin was administered together with diclofenac. The differences in plasma concentration of enrofloxacin and ciprofloxacin in cattle by the above workers as compared to the present study in buffalo calf may be due to differences in physiological and biochemical status between the two species.

*(b) **Kinetic parameters :***

Various kinetic parameters of enrofloxacin obtained when given alone (4 $mg.kg^{-1}$ i.v.) and when given together with diclofenac (1 $mg.kg^{-1}$ i.v.) did not differ significantly (Table 24) which may indicate that diclofenac may not have any influence over distribution, elimination and metabolic processes of enrofloxacin in buffalo calves. Similarly, kinetic parameters of converted ciprofloxacin also did not differ significantly when enrofloxacin was administered alone and when given together with diclofenac. The present findings on comparison of kinetic parameters when the drug was given alone and when given together with diclofenac (Table 24) clearly establish that diclofenac does not have any influence over any physiological, bio-chemical and metabolic processes of enrofloxacin in buffalo calves. In contrast, Verma *et al.* (2000) showed various changes in kinetic parameters such as elimination half life, area under plasma concentration time curve, Vd_{area}, MRT and total body clearance when enrofloxacin given alone (5 $mg.kg^{-1}$ i.m.) or when given together with diclofenac (0.8 to 1 $mg.kg^{-1}$ i.m.). This may be due to species difference and it is well known that physiological status of buffalo widely differ with other ruminants including cattle.

The distribution rate constant (α) of 2.65 ± 0.41 h^{-1} and distribution half life ($t_{½}\ \alpha$) of 0.28 ± 0.04 h were noted for enrofloxacin when administered alone. The values did not differ significantly in

buffalo calves in combined administration of enrofloxacin with diclofenac which denote that similar rate of distribution of the drug occurred in both the groups of animals. A higher $t_{½}$ α of 0.63 to 0.68 h in horse (Giguere *et al.*, 1996), slightly lower $t_{½}$ α of 0.20 ± 0.03 h in goat (Sudha kumari, 1998) and very low $t_{½}$ α of 0.07 ± 0.001 h in chicken (Anadon *et al.*, 1995) while more or less similar $t_{½}$ α of 0.23 ± 0.05 h in pig (Anadon *et al.*, 1999) had been reported after i.v. administration of enrofloxacin.

The elimination rate constant (β) of enrofloxacin and its active metabolite ciprofloxacin were noted be 0.25 ± 0.03 and 0.31 ± 0.05 h^{-1} while the elimination half life ($t_{½}$ β) of 2.92 ± 0.41 and 2.40 ± 0.33 h, respectively following i.v. administration of enrofloxacin (4 $mg.kg^{-1}$) when given alone. The values did not differ significantly in buffalo calves on combined administration of enrofloxacin and diclofenac. This denotes that similar rate of elimination of the drug occurred in both the groups. This is further supported by almost similar value of rate constant of drug for elimination from central compartment (Kel) in buffalo calves when enrofloxacin was given alone and when given together with diclofenac (Table 24). More or less similar $t_{½}$ β of 2.82 ± 0.33 h in goats (Sudha Kumari, 1998), 2.5 h by Broome *et al.* (1991) and 131.5 ± 17.6 min (mean of 2.19 h) by Cabanes *et al.* (1992) in rabbits and 2.4 h (Kung *et al.*, 1993) and 3 h (Kanemaki *et al.*, 1995) in dogs were noted after i.v. administration of

enrofloxacin. In cow, lower $t_{½}$ β of 0.734 h (enrofloxacin) and 0.934 h (ciprofloxacin) by Gardorfer (1991), 0.734 h by Kaartinen *et al.* (1994) and 1.7 h (Kaartinen *et al.*,1995) were noted after i.v. administration of enrofloxacin. In goat lower $t_{1/2}$ β of 0.74 h (enrofloxacin) and 1.38 h (ciprofloxacin) were also noted by Rao *et al.* (2001) after i.m. administration of enrofloxacin. Slightly lower $t_{½}$ β of 1.97 ± 0.23 h in buffalo bulls by Verma *et al.* (1999) was noted after i.m. administration of enrofloxacin. On the other hand higher $t_{½}$ β of 3.73 ± 0.44 h in sheep (Mengozzi *et al.*, 1996), 5.94-6.09 h (Giguere *et al.*,1996), 6.5 h (Zehe, 1990) and 4.4 h (Kaartinen *et al.*,1997) in horse, 5.33 ± 1.05 h in mare (Haines *et al.*, 2000), 17.10 ± 0.09 h in foal (Bermingham *et al.*, 2000), 6.6 h in new born calf and 4.9 h in one week old calf (Kaartinen *et al.*, 1997), 9.64 ± 1.49 h in pig (Anadon *et al.*, 1999) and 10.29 ± 0.45 h (Anadon *et al.*, 1995), 7 h (Knoll *et al.*, 1999) and 4.75 h (Soliman, 2000) in chicken were observed after i.v. administration of enrofloxacin. A high $t_{½}$ β of 4.71 ± 0.67 h for ciprofloxacin in goat (Singh *et al.*, 2001) was found after its single i.v. administration.

The values of rate constant of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment did not differ significantly in buffalo calves when enrofloxacin was given alone and when given together with diclofenac after i.v. administration. The values of K_{12} and K_{21} in buffalo calves when

enrofloxacin given alone were noted to be 1.17 ± 0.27 and 1.06 ± 0.09 h^{-1}, respectively. More or less similar value of K_{12} (Mean value of 0.0216 min^{-1}=1.296 h^{-1}) and K_{21} (0.021 min^{-1} = 1.26 h^{-1}) in rabbits were noted by Cabanes *et al.* (1992). However Giguere *et al.* (1996) noted lower value for K_{12} and K_{21} values (mean ± S.D.) of 0.45 ± 0.62 and 0.54 ± 0.55 h^{-1} were noted in horse. In goat, Sudha Kumari (1998) also noted lower value for K_{12} and K_{21} value of 0.436 ± 0.133 and 0.639 ± 0.087 h^{-1}. In chicken, Anadon *et al.* (1995) reported a very high K_{12} value of 6.13 ± 0.21 h^{-1} and very low K_{21} value of 0.19 ± 0.01 h^{-1} after i.v. administration of enrofloxacin. In the present study almost similar value of rate constant of drug elimination from central compartment (Kel) was observed when enrofloxacin was given alone (0.68 ± 0.15 h^{-1}) as compared to combined administration with diclofenac (0.72 ± 0.19 h^{-1}). Sudha Kumari (1998) reported more or less similar value of Kel (0.577 ± 0.137 h^{-1}) in goats. Giguere *et al.* (1996) noted lower value for Kel of 0.22 ± 0.04 h^{-1} in horse and Anadon *et al.* (1995) reported a very high Kel of 3.46 ± 0.09 h^{-1} in chicken after i.v. administration of enrofloxacin.

The value of area under plasma concentration time curve (AUC) and area under first moment of plasma drug concentration time curve (AUMC) with the mean residential time (MRT) of enrofloxacin as well as its active metabolite ciprofloxacin did not differ significantly between buffalo calves when enrofloxacin was

given alone and when given together with diclofenac (Table 24) The value of AUC, AUMC and MRT of enrofloxacin and its active metabolite ciprofloxacin were noted to be 2.37 ± 0.45 and 1.10 ± 0.27 $mg.L^{-1}$ .h, 7.44 ± 1.67 and 4.29 ± 1.56 $mg.L^{-1}.h^2$ and 3.05 ± 0.20 and 3.47 ± 0.47 h, respectively when enrofloxacin was given alone. More or less similar value of AUC, AUMC and MRT of enroflxacin and its active metabolite ciprofloxacin were noted in buffalo calves when enrofloxacin was given together with diclofenac following i.v. administration of enrofloxacin. A very high value of AUC of 48.54 ± 10.46 (mean ± S.D.) in foal by Bermingham *et al.* (2000) and 21.03 ± 0.19 in mare by Haines *et al.* (2000) were noted after i.v. administration of enrofloxacin. A very low value of MRT of 1.55 h in rabbits (Broome *et al.*, 1991), a very high value of MRT of 12.77 ± 2.15 h in pig (Anadon *et al.*, 1999) and 9.0 h (Knoll *et al.*,1999), 6.72 h (Soliman, 2000) in chicken were noted after i.v. administration of enrofloxacin. A high value of AUC of 7.11 ± 1.73 $mg.L^{-1}.h$ in goat (Singh *et al.*, 2001) was noted after a single i.v. administration of ciprofloxacin.

The kinetic parameters namely fraction of drug available for elimination from central compartment (Fc), approximate tissue to plasma concentration ratio (T≈P), various values of volume of distribution, total body clearance (Cl_B) and % conversion of enrofloxacin to ciprofloxacin (AUC_{cipro}/AUC_{enro}) also did not differ

significantly between buffalo calves when enrofloxacin was given alone and on combination with diclofenac (Table 24).

Notari (1980) stated that for a two-compartment open model, the value of Vd_B > Vd_{area}> Vd_{SS} and Vd_C . He further mentioned that among these values of volume of distribution, only Vd_{area} correctly predicts the amount of drug in the body during elimination phase where as Vd_B over estimates and Vd_{SS} & Vd_C under estimate the amount of drug in the body. Similarly, the value of Vd_{SS} obtained by non-compartmental model, correctly predict the amount of drug and/or its metabolite in the body during elimination phase (Bhupinder Singh and Naveen Ahuja, 1999). Vd_{area} of 7.47 ± 0.69 $L.kg^{-1}$ was obtained for enrofloxacin while Vd_{SS} of enrofloxacin and its active metabolite ciprofloxacin 5.33 ± 1.04 and 13.60 ± 1.23 $L.kg^{-1}$, respectively were obtained in the present study after i.v. administration of enrofloxacin alone. Volume distribution of 0.6 $L.kg^{-1}$ (Gardorfer, 1991 and Kaartinen *et al.*, 1994) and 1 $L.kg^{-1}$ (Kaartinen *et al.*, 1995) in cow, 1.8 $L.kg^{-1}$ in new born calf and 2.3 $L.kg^{-1}$ in one week old calf (Kaartinen *et al.*, 1997), 0.61 ± 0.13 $L.kg^{-1}$ in buffalo bull (Verma *et al.*, 1999), 2 $L.kg^{-1}$ (Zehe, 1990), 0.77 ± 0.11 - 1.22 ± 0.07 $L.kg^{-1}$ Giguere *et al.*,1996) and 2.3 $L.kg^{-1}$ (Kaartinen *et al.*, 1997) in horse, 2.49 ± 0.43 $L.kg^{-1}$ in foal (Bermingham *et al.*, 2000), 3.02 ± 0.22 $L.kg^{-1}$ in sheep (Mengozzi *et al.*, 1996), 2.34 ± 0.54 $L.kg^{-1}$ (Sudha Kumari, 1998) and 1.42 $L.kg^{-1}$ (Rao *et al.*, 2001) in goat, 7 $L.kg^{-1}$ in dog

(Kung *et al.*,1993), 2.12 L.kg^{-1} (Broome *et al.*, 1991) and 3.4 ± 0.9 L.kg^{-1} (Cabanes *et al.*, 1992) in rabbit, 4.31 ± 0.15 L.kg^{-1} (Anadon *et al.*, 1995), 1.98 ± 0.18 L.kg^{-1} (enrofloxacin) and 4.04 ± 0.69 L.kg^{-1} (ciprofloxacin) by Garcia-Ovando *et al.* (1999), 4 L.kg^{-1} (Knoll *et al.*,1999) and 1.11 L.kg^{-1} (Soliman, 2000) in chicken were reported. The above findings denote that enrofloxacin is well distributed in different tissues and body fluids in the above species of animals including buffalo calves.

The total body clearance (Cl_B) values of enrofloxacin and its active metabolite ciprofloxacin did not differ significantly between buffalo calves when enrofloxacin was given alone and when given in combination with diclofenac after i.v. administration (Table 24). Cl_B value of enrofloxacin and its active metabolite ciprofloxacin were noted to be 32.40 ± 5.69 and 72.38 ± 13.17 ml. kg^{-1}.min^{-1}, respectively after i.v. administration of enrofloxacin. More or less similar Cl_B value of 27.1 ml.kg^{-1}.ml^{-1} was noted for enrofloxacin in dog (Kung *et al.*, 1993). However, lower Cl_B value of 22.8 ± 6.8 ml.kg^{-1}.min^{-1} in rabbit (Cabanes *et al.*,1992), 22.11 ml.kg^{-1}.min^{-1} in goat (Rao *et al.*, 2001), very low Cl_B value of 9.17 ± 2.4 ml.kg^{-1}.min^{-1} in sheep (Mengozzi *et al.*,1996), 1.50 ± 2.33 ml.kg^{-1}.min^{-1} in horse (Giguere *et al.*, 1996), 1.73 ml.kg^{-1}.min^{-1} in foal (Bermingham *et al.*, 2000), 3.17 ml.kg^{-1}.min^{-1} in new born calf and 6.5 ml.kg^{-1}.min^{-1} in one week old calf (Kaartinen *et al.*, 1997), 9.40 ± 1.36 ml. kg^{-1}.ml^{-1} in goat (Sudha Kumari, 1998),

4.83 ml.kg^{-1}.min^{-1} (Anadon *et al.*, 1995), 10 ml. kg^{-1}.min^{-1} (Knoll *et al.*, 1999) and 5.83 ml.kg^{-1}.min^{-1} (Soliman, 2000) in chicken were obtained after i.v. administration of enrofloxacin where as, a very low Cl_B value of 11.19 ± 1.55 ml.kg^{-1}.min^{-1} was noted in goat (Singh *et al.*, 2001) after a single i.v. administration of ciprofloxacin.

The percentage conversion of enrofloxacin to ciprofloxacin (AUC_{cipro}/AUC_{enro}) did not differ significantly between buffalo calves when enrofloxacin was given alone (46.96 ± 5.60) and when given in combination with diclofenac (42.17 ± 9.85) after i.v. administration. A slightly lower value of the metabolic conversion of enrofloxacin to ciprofloxacin (36%) was noted in goats by Rao *et al.* (2001) after i.m. administration of enrofloxacin.

c. Urinary excretion :

Concentrations of enrofloxacin (including its active metabolite ciprofloxacin) in urine estimated by microbiological assay were noted to differ only non-significantly in buffalo calves when enrofloxacin (4 mg.kg^{-1}) was given alone and when given together with diclofenac (1 mg.kg^{-1}) following i.v. administration (Table 25 and Fig. 3). In contrast, when enrofloxacin was given along with paracetamol, concentrations of enrofloxacin in urine were observed to be significantly higher initially (5 to 30 min) and later (5 to 48 h) as compared to single administration of enrofloxacin in goat for i.v. route (Sudha Kumari, 1998). Peak concentration in urine was achieved

earlier at 0.333 h when given together with diclofenac as compared to 0.50 h when enrofloxacin was given alone. The minimum therapeutic concentration (≥ 0.125 μg. ml^{-1}) in urine was maintained from 0.042 to 48 h for both the groups of animals in the present study. Similar is the observation of Sudha Kumari (1998) when enrofloxacin was given alone and when given together with paracetamol in goats by i.v. route.

d. Dosage regimen :

The minimum inhibitory concentration (MIC) values of enrofloxacin for different species of microorganism ranged between 0.001 to 1.0 μg.ml^{-1} in veterinary practice (Mevius *et al.*, 1990 ; Prescott and Yielding, 1990). It is known that sensitivity or resistant of microorganisms to enrofloxacin is more or less similar to its close congener, ciprofloxacin. Raina (1991) and Singh *et al.* (2001) calculated dosage regimen of ciprofloxacin by taking 0.12 μg.ml^{-1} as therapeutic concentration (MIC). Similarly, Uday Kumar (2000) had taken 0.12 μg.ml^{-1} as MIC for calculating dosage regimen of enrofloxacin.

No significant difference in loading (D*) and maintenance (D_0) doses was observed when enrofloxacin was given alone and when given together with diclofenac by i.v. route (Table 26) which suggests that diclofenac may not have any influence in altering doses of enrofloxacin and can be combined safely for effective therapy of microbial infections accompanied by inflammatory conditions.

Kinetic study of diclofenac :

Kinetic studies of diclofenac in animals are very little and studies in man (Willis *et al.*, 1979 ; Kurowski, 1988), pig (Oberle *et al.*, 1994) and rat (Peris-Ribera *et al.*, 1991) were reported.

a. Distribution in plasma :

Concentrations of diclofenac in plasma were found to be significantly lower initially form 0.042 to 3 h and significantly higher later from 8 to 24 h in buffalo calves when administered in combination with enrofloxacin as compared to single administration of diclofenac by i.v route (Table 27 and Fig. 4). Available literature shows that no kinetic study including plasma levels of diclofenac was studied in domestic species so far.

b. Kinetic parameters :

A significantly lower value for the extrapolated zero time concentration during distribution phase (A), a non-significantly slightly lower value for the extrapolated zero time concentration during elimination phase (B) and a significantly lower value for theoretical zero time concentration (C_p^0) for diclofenac when administered in combination with enrofloxacin as compared to single administration of diclofenac by i.v. route (Table 28).

The distribution rate constant (α) of 2.76 ± 0.81 h^{-1} and distribution half life ($t_{½}$ α) of 0.34 ± 0.08 h were calculated for diclofenac when given alone by i.v. route. The values did not differ significantly in buffalo calves when combined i.v. administration of

diclofenac with enrofloxacin denoting similar rate of distribution of drug occurred in buffalo calves between both the groups. The shorter $t_{1/2}$ α denotes that the drug is comparatively distributed at a faster rate in buffalo calves.

The elimination rate constant (β) of 0.19 ± 0.03 h^{-1} and elimination half life ($t_{1/2}$ β) of 4.06 ± 0.59 h estimated after single i.v. administration of diclofenac. Significantly decreased β (0.06 ± 0.01 h^{-1}) and highly increased $t_{1/2}$ β (12.84 ± 1.29 h) were noted for diclofenac when it was given in combination with enrofloxacin by i.v. route. The increased $t_{1/2}$ β observed after combined i.v. administration of diclofenac with enrofloxacin indicates very slow removal of the drug from the body as compared to single administration of diclofenac. This is further supported by lower value of rate constant of drug elimination from central compartment (Kel) obtained in buffalo calves (0.14 ± 0.01 h^{-1}) after combined i.v. administration of diclofenac with enrofloxacin. The $t_{1/2}$ β value of 1.1 h in man after i.v administration of diclofenac (Willis *et al.*, 1979) and 1.15 h in man after i.m. injection of diclofenac (Kurowski, 1988) were noted to be very low than the value obtained in buffalo calves in the present study. The terminal half life ($t_{1/2}$ β) of diclofenac were similar in pigs (2.4 h) and man (1.8 h) as observed by Oberle *et al.* (1994).

The values of rate of transfer of drug from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment did not differ significantly between buffalo calves when diclofenac was given

alone and when given together with enrofloxacin by i.v. route (Table 28). Similarly, some of the other kinetic parameters namely fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration (T≈P) did not differ significantly between buffalo calves when diclofenac was given alone and when given together with enrofloxacin after i.v. administration.

The values of area under plasma concentration time curve (AUC) was noted to be 11.24 ± 0.48 $mg.L^{-1}.h$ when diclofenac (1 $mg.kg^{-1}$) was given alone in buffalo calves following i.v. administration. This value did not differ significantly in buffalo calves when diclofenac was given together with enrofloxacin (4 $mg.kg^{-1}$) after i.v. administration. The value of total area under the first moment of plasma drug concentration time curve (AUMC) in buffalo calves after i.v. administration was noted to be 51.78 ± 7.30 $mg.L^{-1}.h^2$ when diclofenac was given alone. This value noted to differ significantly lower than those obtained when diclofenac was given together with enrofloxacin in buffalo calves (264.8 ± 58.10 $mg.L^{-1}.h^2$) after i.v. administration. The value of mean residential time (MRT) in buffalo calves after i.v. administration was noted to be 4.72 ± 0.85 h when diclofenac was given alone which was significantly lower ($P<0.01$) than those obtained when diclofenac was given together with enrofloxacin in buffalo calves (18.07 ± 1.92 h) after i.v. administration. Significantly higher values of AUMC and MRT in

buffalo calves when diclofenac was given together with enrofloxacin reflect that the drug remains in the body for comparatively longer duration in combination with enrofloxacin.

The various values of volume of distribution were significantly higher in buffalo calves when diclofenac was given together with enrofloxacin as compared to single administration of diclofenac after i.v. administration (Table 28). Vd_{area} of 0.54 ± 0.10 $L.kg^{-1}$ was noted for single administration of diclofenac by i.v. route. A very low value of volume of distribution 0.17 ± 0.11 $L.kg^{-1}$ in man (Willis *et al.*, 1979) and the volume of distribution of the central compartment (Vd_C) was 40% less in man than in pigs (0.039 vs 0.067 $L.kg^{-1}$) as noted by Oberle *et al.* (1994).

The total body clearance (Cl_B) values did not differ significantly between buffalo calves when diclofenac was given alone and when given together with enrofloxacin by i.v. route. Cl_B value of 1.52 ± 0.07 $ml.kg^{-1}.min^{-1}$ was observed after single i.v. administration of diclofenac. A high Cl_B value of 4.2 ± 0.9 $ml.kg^{-1}.min^{-1}$ in man (Willis *et al.*, 1979) was observed. The total plasma clearance (Cl_B) in minipigs was five fold slower than in man (57 ± 17 $ml.kg^{-1}.h^{-1}$ = 0.95 ± 0.28 $ml.kg^{-1}.min^{-1}$ vs 252 ± 54 $ml.kg^{-1}.h^{-1}$ = 4.2 ± 0.9 $ml.kg^{-1}.min^{-1}$) as noted by Oberle *et al.* (1994). The value obtained by Oberle *et al.* (1994) in minipigs were more or less similar to buffalo calves noted in present study.

c. Urinary excretion :

Concentrations of diclofenac in urine were noted to be significantly lower initially from 0.167 to 1.5 h and significantly higher later from 4 to 48 h in buffalo calves when diclofenac was given together with enrofloxacin as compared to single administration of diclofenac by i.v. route (Table 27 and Fig. 5). Peak concentration in urine was noted earlier at 0.167 h in case of single administration of diclofenac as compared to 4 h noted in case of combined administration of diclofenac with enrofloxacin by i.v. route. Urinary excretion less than 1% in man observed by Willis *et al.* (1979).

Kinetic interactions between enrofloxacin and diclofenac :

The distribution of enrofloxacin and diclofenac in plasma and urine as well as various kinetic parameter have been described above when given alone or in combination following i.v. administration. Definite kinetic interactions between the drugs occurred in buffalo calves and the salient features are described below :

The results of the present study clearly establishes that diclofenac does not have any influence over kinetics of enrofloxacin as well as its active metabolite ciprofloxacin which results in similar calculated loading (D^*) and maintenance (D_0) doses when enrofloxacin was given alone or when administered together with diclofenac. The above statements leads to the inference that

enrofloxacin can be used effectively along with diclofenac in clinical cases of drug sensitive microbial infections accompanied by any other inflammatory conditions.

In contrast, enrofloxacin may influence over diclofenac as noted by significant changes in plasma and urine levels as well as on various kinetic parameters (Tables 27 and 28). Since enrofloxacin has increased the elimination half life ($t_{½}$ β), MRT and Vd_{area} which may be beneficial under inflammatory conditions since the drug may be distributed in greater amount in body tissues and remain for longer time when diclofenac was administered together with enrofloxacin as compared to its single administration.

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Chapter – VI

Summary

SUMMARY

A detailed pharmacokinetic study of enrofloxacin and diclofenac when given alone and their interactions when given in combination was carried out in buffalo calves weighing between 102-175 kg post i.v. administration. Concentrations of the drugs in plasma and urine as well as various kinetic parameters were calculated by using appropriate compartment models when given alone or when given together. Attempts were made to calculate the rational dosage regimen of enrofloxacin on the basis of kinetic data and maintenance of therapeutic concentrations (MIC) in plasma. The findings were as follows :

1. Following combined i.v. administration of enrofloxacin (4mg.kg^{-1}) with diclofenac (1 mg.kg^{-1}) plasma concentrations of enrofloxacin were non-significantly different between when given alone or when given together with diclofenac. Similarly, no significant difference between plasma concentrations of ciprofloxacin (active metabolite of enrofloxacin) following i.v. administration in buffalo calves when enrofloxacin was given alone and when given together with diclofenac. Peak plasma concentration of ciprofloxacin was achieved earlier at 0.25 h when enrofloxacin was given alone and at 0.333 h when given together with diclofenac. In case of urine, the concentrations of enrofloxacin

(including its active metabolite ciprofloxacin) estimated by microbiological assay were noted to differ non-significantly between both the groups of animal. The minimum therapeutic concentration (≥ 0.125 $\mu g.ml^{-1}$) in urine was maintained from 0.042 to 48 h between both the groups of animal (Table 25).

Following combined i.v. administration of diclofenac with enrofloxacin, concentrations of diclofenac in plasma were found to be significantly lower initially (0.042 to 3 h) and significantly higher later (8 to 24 h) as compared to single i.v. administration of diclofenac. In case of urine samples, the concentrations of diclofenac were found to be significantly lower initially (0.167 to 1.5 h) and significantly higher later (4 to 48 h)in buffalo calves when diclofenac was given together with enrofloxacin as compared to single administration of diclofenac. Peak concentration of diclofenac in urine was noted at 0.167 h when diclofenac given alone as compared to 4 h incase of combined administration of diclofenac with enrofloxacin (Table 27).

2. Various kinetic parameters of enrofloxacin as well as its active metabolite ciprofloxacin did not differ significantly when enrofloxacin was administered alone or in combination with diclofenac. The above noted results show that diclofenac may not have any influence over kinetics of enrofloxacin and there by

does not affect its distribution and elimination in buffalo calves. This may be the reason that the calculated dosage regimen of enrofloxacin did not differ significantly when given alone or in combination with diclofenac (Table 24).

The percentage conversion of enrofloxacin to ciprofloxacin was 46.96 ± 5.60 when enrofloxacin was given alone as compared to 42.17 ± 9.85 on combined administration of enrofloxacin with diclofenac in buffalo calves but the difference was non-significant. This shows that the metabolism of enrofloxacin was not at all influenced by diclofenac in buffalo calves.

3. The distribution rate constant (α) and distribution half life ($t_{1/2}$ α) did not differ significantly for enrofloxacin and diclofenac when administered alone or in combination which denote that similar rate of distribution of these drugs occurred in buffalo calves after i.v. administration when given alone or given together.

4. Significantly lower elimination rate constant (β) and higher elimination half life ($t_{1/2}$ β) obtained after combined i.v. administration of diclofenac with enrofloxacin as compared to single administration of diclofenac indicate slower elimination of diclofenac when given together with enrofloxacin. This is further

supported by lower value of rate constant of drug elimination from central compartment (Kel) obtained for diclofenac when given together with enrofloxacin (Table 28).

5. The rate constant of drug transfer from central to peripheral compartment (K_{12}), peripheral to central compartment (K_{21}), fraction of drug available for elimination from central compartment (Fc), approximate tissue to plasma concentration ratio (T≈P) and area under plasma concentration time curve (AUC) were observed to be non-significant for enrofloxacin and diclofenac when administered alone or in combined administration in buffalo calves.

6. Significantly higher value of AUMC and MRT of 264.8 ± 58.10 mg. L^{-1}. h^2 and 18.07 ± 1.92 h for diclofenac were obtained when it was given together with enrofloxacin as compared to 51.78 ± 7.30 mg. $L^{-1}.h^2$ and 4.72 ± 0.85 h, respectively when diclofenac was given alone in buffalo calves following i.v. administration.

7. Various values of volume of distribution were found to differ significantly for diclofenac after i.v. administration in buffalo calves when diclofenac was given alone as compared to combined administration of diclofenac with enrofloxacin. The values of Vd_{area} of 1.34 ± 0.04 $L.kg^{-1}$ when diclofenac given together with

enrofloxacin was significantly higher as compared to 0.54 ± 0.10 $L.kg^{-1}$ when diclofenac was given alone after i.v. administration in buffalo calves. High Vd_{area} value denotes good distribution of diclofenac in different tissues and body fluids on combined administration of diclofenac with enrofloxacin as compared to single administration of diclofenac in buffalo calves. Total body clearance (Cl_B) values did not differ significantly for diclofenac and enrofloxacin when given alone or in combination following i.v. administration.

8. By taking into account the maintenance of therapeutic concentration in plasma, enrofloxacin may be administered at the dose rate of 6 $mg.kg^{-1}$ as loading dose (D*) and 5 $mg.kg^{-1}$ as maintenance dose (D_0) at the dosage interval (γ) of 8 h for treating septicaemia and other systemic infections when administered alone. When given in combination with diclofenac, for treating the above conditions accompanied by inflammatory conditions, the drug should be given at the same dose rate and same dosage interval, which may be safe and effective.

The present study clearly establishes that diclofenac does not have any influence over kinetics of enrofloxacin which results in similar calculated loading (D*) and maintenance (D_0) doses when enrofloxacin was given alone or when administered together with

diclofenac. The above statements lead to the inference that enrofloxacin can be used effectively along with diclofenac in clinical cases of drug sensitive microbial infections accompanied by any other inflammatory conditions.

In contrast, enrofloxacin may influence over kinetics of diclofenac as noted by significant changes in plasma and urine levels as well as on various kinetic parameters. Since enrofloxacin has increased the elimination half life, MRT and Vd_{area} which may be beneficial under inflammatory conditions. The drug may be distributed in greater amount in body tissues and remain for longer time when diclofenac was administered together with enrofloxacin as compared to its single administration. Further studies should be carried out to know the amount of distribution of diclofenac in tissues, an body fluids like synovial fluid, bronchial tissues, secretions of various organs etc. under inflammatory conditions when antimicrobials (including enrofloxacin) were given together in clinical practice.

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Appendix

APPENDIX – I

CALCULATION OF KINETIC PARAMETERS :

Kinetic parameters were calculated from the plasma log drug concentration versus time profile. An example is noted below from the data of animal no.3 obtained after i.v. injection of enrofloxacin (4 $mg.kg^{-1}$) given alone in buffalo calf. The data showed a biphasic curve and hence, fits well into two-compartment open model. Here, elimination phase starts from 1.5 h.

No.	Time (h) X	X^2	Plasma concentration Y ($\mu g.\ ml^{-1}$)	Log (Y×10)	XY
1.	1.5	2.25	0.20	0.3010	0.4515
2.	2	4	0.17	0.2304	0.4609
3.	3	9	0.13	0.1139	0.3418
4.	4	16	0.10	0.0000	0.0000
5.	5	25	0.08	-0.0969	0.4846
6.	6	36	0.06	-0.2218	-1.3311
7.	8	64	0.04	-0.3979	-3.1835
8.	10	100	0.02	-0.6990	-6.9900
9.	12	144	0.01	-1.0000	-12.000

$n = 9$ $\Sigma X = 51.5$ $\Sigma X^2 = 400.25$ $\Sigma Y = -1.7703$ $\Sigma XY = -22.735$

$\bar{x} = 5.72$ $(\Sigma X)^2 = 2652.25$ $\bar{Y} = -0.1967$

$$\text{b, slope of line} = \frac{n.\Sigma XY - \Sigma X.\Sigma Y}{n.\Sigma X^2 - (\Sigma X)^2}$$

where, X= time ; Y = drug concentration ; n= number of samples.

$$b = \frac{9 \times (-22.735) - 51.5 \times (-1.7703)}{9 \times 400.25 - 2652.25}$$

$$= \frac{-204.615 - (-91.1705)}{3602.25 - 2652.25}$$

$$= \frac{-113.4445}{950} = -0.1194$$

β, elimination rate constant $= b \times -2.303$

$= -0.1194 \times (-2.303)$

$= 0.275 \approx 0.28\ h^{-1}$

B, zero time concentration during elimination phase can be obtained from the formula :

$$\overline{Y} = a + b\overline{X}$$

where, $\overline{Y}$ = mean log concentration

$\overline{X}$ = mean time

b = slope of line

a = zero time concentration

Therefore, $a = \overline{Y} - b\overline{X}$

$= \log - 0.1967 - (-0.1194 \times 5.72)$

$= \log - 0.1967 - (-0.683)$

$= \log - 0.1967 + 0.683$

$= \log 0.4863$

Zero time concentration = antilog of 0.4863 = 3.06 $\mu g.ml^{-1}$

Since plasma concentration is multiplied earlier by 10 in the above calculation, the value of 3.06 $\mu g.ml^{-1}$ should be divided by 10 to get the actual zero time concentration. Hence, zero time concentration (B) = 0.306 $\mu g.ml^{-1}$ or 0.31 $\mu g.ml^{-1}$.

Similarly, the theoretical plasma concentration (Y) can be calculated by putting the values of time (X) in the above equation during the time intervals of distribution phase (Y = a+bX).

Subtracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and zero time intercept (zero time concentration during distribution phase, A) can be calculated as per the method adopted for calculation of B and β. The calculated values are –

$\alpha =$ 2.73 h^{-1}

A= 1.05 $\mu g.\ ml^{-1}$

C_p^0, theoretical plasma concentration at zero time

$$C_p^0 = A+B$$

$$= 1.05+0.31 = 1.36\ \mu g.\ ml^{-1}$$

$t_{1/2}\alpha$, distribution half life

$$t_{1/2}\alpha = \frac{0.693}{\alpha} = \frac{0.693}{2.73} = 0.25h$$

$t_{1/2}\beta$, elimination half life

$$t_{1/2\beta} = \frac{0.693}{\beta} = \frac{0.693}{0.28} = 2.48h$$

AUC, area under curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

$$= \frac{1.05}{2.73} + \frac{0.31}{0.28} = 0.38 + 1.11 = 1.49\ mg.L^{-1}.h$$

AUMC, area under the first moment of plasma drug concentration time curve.

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

$$= \frac{1.05}{7.4529} + \frac{0.31}{0.0784}$$

$$= 0.14 + 3.95 = 4.09\ mg.L^{1}.h^{2}$$

MRT, mean residential time

$$MRT = \frac{AUMC}{AUC} = \frac{4.09}{1.49} = 2.74\ h$$

K_{21}, rate constant for drug transfer from pe4ripheral to central compartment.

$$K_{21} = \frac{A.\beta + B.\alpha}{C_p^0} = \frac{1.05 \times 0.28 + 0.31 \times 2.73}{1.36} = 0.84h^{-1}$$

Kel, the elimination rate constant of the drug from central compartment.

$$Kel = \frac{\alpha.\beta}{K_{21}} = \frac{2.73 \times 0.28}{0.84} = 0.91 h^{-1}$$

K_{12}, rate constant of drug transfer from central to peripheral compartment.

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

$$= 2.73 + 0.28 - 0.84 - 0.91 = 1.26\ h^{-1}$$

Fc, the fraction of drug available for elimination from central compartment.

$$Fc = \frac{\beta}{Kel} = \frac{0.28}{0.91} = 0.31$$

$T \approx P$, approximate tissue to plasma concentration ratio.

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{1.26}{0.84 - 0.28} = \frac{1.26}{0.56} = 2.25$$

Vd_C, the volume of distribution based on distribution and elimination.

$$Vd_C = \frac{D}{A + B}, \text{ where D= dose rate } (mg.kg^{-1})$$

$$= \frac{4}{1.05 + 0.31} = 2.94\ L.kg^{-1}$$

Vd_B, the volume of distribution based on elimination

$$Vd_B = \frac{D}{B} = \frac{4}{0.31} = 12.90\ L.kg^{-1}$$

Vd_{area}, the volume of distribution based on total area under curve.

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{4}{1.49 \times 0.28} = 9.59\ L.kg^{-1}$$

Vd_{SS}, the volume of distribution at steady state.

$$Vd_{ss} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd_C$$

$$= \frac{1.26 + 0.84}{0.84} \times 2.94 = 7.35\ L.kg^{-1}$$

Cl_B, the total body clearance

$$Cl_B = Vd_{area} \times \beta$$

$$= 9.59 \times 0.28 = 2.69\ L.kg^{-1}.h^{-1}$$

$$= 44.83\ ml.kg^{-1}.min^{-1}$$

APPENDIX – II

NON-COMPARTMENTAL PHARMACOKINETIC ANALYSIS OF PLASMA LEVEL DATA THROUGH STATISTICAL MOMENT APPROACH :

Calculation of kinetic parameters of ciprofloxacin (active metabolite of enrofloxacin) from plasma level data at various time intervals. An example is mentioned here from the data of animal no. 3 obtained after i.v. injection of enrofloxacin (4 $mg.kg^{-1}$) given alone in buffalo calf (Slope of terminal line, $\lambda=0.52\ h^{-1}$).

Time(h) (t)	Conc. (C) $\mu g.ml^{-1}$	delta t (Δt) (h)	C_{bar} $\mu g.ml^{-1}$	C.t $\mu g.ml^{-1}.h$	$C.t_{bar}$ $\mu g.ml^{-1}.h$	$AUT=C_{bar}.\Delta t$ $\mu g.ml^{-1}.h$ or $mg.L^{-1}.h$	$AUMT=Ct_{bar}.\Delta t$ $mg.L^{-1}.h^2$
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.042	0.03	0.042	0.015	0.0012	0.0006	0.0006	0.00002
0.083	0.11	0.041	0.07	0.0091	0.0051	0.0029	0.0002
0.167	0.24	0.084	0.175	0.04	0.0245	0.0147	0.0021
0.25	0.25	0.083	0.245	0.0625	0.0512	0.0203	0.0042
0.333	0.28	0.083	0.265	0.093	0.0778	0.022	0.0065
0.50	0.31	0.167	0.295	0.155	0.124	0.0493	0.0207
0.75	0.23	0.25	0.27	0.1725	0.1637	0.0675	0.0409
1	0.18	0.25	0.205	0.18	0.1762	0.0512	0.044
1.5	0.15	0.5	0.165	0.225	0.2025	0.0825	0.1013
2	0.13	0.5	0.14	0.26	0.2425	0.07	0.1213
3	0.05	1	0.09	0.15	0.205	0.09	0.205
4	0.03	1	0.04	0.12	0.135	0.04	0.135
5	0.03	1	0.03	0.15	0.135	0.03	0.135
6	0.02	1	0.025	0.12	0.135	0.025	0.135
8	0.01	2	0.015	0.08	0.1	0.03	0.2
Total areas under the curve upto last sampling time						$\Sigma AUT(AUC)$ = 0.596	$\Sigma AUMT(AUMC)$ = 1.151

MRT = AUMC/AUC = 1.151/0.596 = 1.93 ; $t_{1/2}\lambda = 0.693 \times 1.93 = 1.34$;

Slope of terminal line, $\lambda = 0.693 / 1.34 = 0.52\ h^{-1}$

1. Area under curve upto last sampling (AUC_{t^*})= 0.596 $mg.L^{-1}.h$

2. Terminal AUC beyond last sampling ($AUC_{t^*-\infty}= c^*/\lambda$)

$$= \frac{0.01}{0.52} = 0.02 mg.L^{-1}.h$$

3. Total AUC ($AUC_\infty = AUC_{t^*} + AUC_{t^*-\infty}$)

$$= 0.596 + 0.02 = 0.616 \cong 0.62\ mg.L^{-1}.h.$$

4. Total body clearance

$$\left(Cl_B = \frac{X_0}{AUC_\infty}\right) = \frac{4}{0.62} = 6.45\ L.kg^{-1}.h = 107.53\ ml.kg^{-1}.min^{-1}$$

5. Area under moment curve upto last sampling ($AUMC_{t^*}$)

$$= 1.151\ mg.L^{-1}.h^2$$

6. Terminal AUMC beyond last sampling

$$\left[AUMC_{t^*-\infty} = \frac{C^*.t^*}{\lambda} + \left(\frac{C^*}{\lambda}\right)^2\right] = \frac{0.01 \times 8}{0.52} + \left(\frac{0.01}{0.52}\right)^2$$

$$= 0.154\ mg.L^{-1}.h^2$$

7. Total AUMC ($AUMC_\infty = AUMC_{t^*} + AUMC_{t^*-\infty}$)

$$= 1.151 + 0.154 = 1.305 \cong 1.31\ mg.L^{-1}.h^2$$

8. Mean residential time $\left(MRT = \frac{AUMC_\infty}{AUC_\infty}\right) = \frac{1.31}{0.62} = 2.11\ h$

9. Elimination half life ($t_{1/2}\beta = 0.693 \times MRT$) = $0.693 \times 2.11 = 1.46\ h$

10. Apparent overall first-order elimination rate constant

$$\left(k \text{ or } \beta = \frac{1}{MRT}\right) = \frac{1}{2.11} = 0.47\ h^{-1}$$

f distribution

$$\left(\frac{Cl_B}{\beta}\right) = \frac{6.45}{0.47} = 13.72\ L.kg^{-1}$$

cin to ciprofloxacin (AUC_{cipro}/AUC_{enro})

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11. Steady state volume of distribution

$$\left(Vd_{SS} = \frac{Cl_B}{\beta}\right) = \frac{6.45}{0.47} = 13.72\ L.\,kg^{-1}$$

12. % conversion of enrofloxacin to ciprofloxacin (AUC_{cipro}/AUC_{enro})

$$= \frac{0.62}{1.49} = 41.61$$

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APPENDIX – III

Dosage regimen were calculated to maintain the desired levels of therapeutic concentration (MIC) in plasma at desired dosage intervals using the formulae described by Saini and Srivastava (1997). The data of animal no.3 obtained for enrofloxacin + ciprofloxcacin together needed for calculation of dosage regimen of enrofloxacin after i.v. administration in buffalo calf has been used as an example for calculation of dosage regimen for maintaining MIC (C_p^{∞} min) of 0.125 $\mu g.ml^{-1}$ at the dosage interval (γ) of 8 h. The calculation is as follows :

Calculation of loading or priming dose (D*) :

For calculation of D, the following formula is used :*

$$D^* = C_p^{\infty}(min).Vd_{area}\,(e^{\beta.\gamma})$$

Where, β = Elimination rate constant

γ = Dosage interval

e = Base of natural logarithm.

for $C_p^{\infty}(min)$ of 0.125 $\mu g.ml^{-1}$ and γ of 8 h

$$D^* = 0.125 \times 6.04\,(e^{0.32 \times 8}) = 9{:}76\ mg.kg^{-1}$$

Calculation of maintenance dose (D_0) :

For calculating D_0, the following formula is employed :

$$D_0 = C_p^{\infty}(min).\,Vd_{area}\,(e^{\beta.\gamma}-1)$$

$$= 0.125 \times 6.04 \times (e^{0.32 \times 8}-1) = 9.01\ mg.kg^{-1}.$$

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