"STUDIES ON EFFECT OF TOLFENAMIC ACID AND BIOENHANCER TRIKATU ON PHARMACOKINETICS OF CEFQUINOME AND SAFETY OF SIMULTANEOUS ADMINISTRATION OF CEFQUINOME AND TOLFENAMIC ACID IN PATANWADI SHEEP"

A
THESIS
SUBMITTED TO THE
ANAND AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

OF

Master of Veterinary Science

IN

(VETERINARY PHARMACOLOGY & TOXICOLOGY)

BY

MAYANKKUMAR PRABHUBHAI RANA
B.V.Sc. & A.H.
(Reg. No. 04-1724-2011)

DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY
COLLEGE OF VETERINARY SCIENCE & ANIMAL HUSBANDRY
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ANAND-388 001

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RANA MAYANKKUMAR PRABHUBHAI
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(Reg. No. 04-1724-2011)

DEPARTMENT OF VETERINARY PHARMACOLOGY & TOXICOLOGY
COLLEGE OF VETERINARY SCIENCE & ANIMAL HUSBANDRY
ANAND AGRICULTURAL UNIVERSITY
ANAND - 388001(GUJARAT)

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ABSTRACT

"STUDIES ON EFFECT OF TOLFENAMIC ACID AND BIOENHANCER TRIKATU ON PHARMACOKINETICS OF CEFQUINOME AND SAFETY OF SIMULTANEOUS ADMINISTRATION OF CEFQUINOME AND TOLFENAMIC ACID IN PATANWADI SHEEP"

Name of Student	**Name of Major Advisor**
Mayankkumar P. Rana	**Dr. A. M. Thaker**

Department of Veterinary Pharmacology and Toxicology
College of Veterinary Science and Animal Husbandry
Anand Agricultural University
Anand 388 001
Gujarat, INDIA

Antibacterials are frequently recommended as an adjunct therapy with non-steroidal anti-inflammatory drugs (NSAIDs) to treat various bacterial infections and inflammatory conditions in animals. Cefquinome is a fourth-generation cephalosporin, which has been developed solely for veterinary use. It shows potent antibacterial activity against a broad spectrum of bacterial species including those that are resistant to conventional antibacterial drug. Tolfenamic acid (TA) is a new NSAID having anti-inflammatory, analgesic and antipyretic properties. In veterinary practice, tolfenamic acid is used clinically as an anti-inflammatory and analgesic agent for the therapy of locomotor diseases in the dog and post-operative pain in cats. Pharmacokinetics of an antibacterial drug may change when administered with anti-inflammatory drug. Despite the potential for clinical use of cefquinome, the data on its pharmacokinetics interaction and safety profile in sheep are not available. Ancient and recent scientific literature cited reference of bioenhancer like trikatu application to increase bioavailability of drug and nutrients. Moreover, it is well known in ruminant animals that oral bioavailability of drug is low as compared to monogastric animals.

Looking to this, present study was conceptualized to determine the effect of tolfenamic acid (2 mg/kg body weight) on pharmacokinetics of cefquinome

(2 mg/kg body weight) following its intramuscular administration in sheep and safety of daily intramuscular administration of cefquinome (2 mg/kg body weight) in combination with tolfenamic acid (2 mg/kg body weight) for five days in sheep by monitoring hematological and blood biochemical profiles. Moreover, effect of bioenhancer trikatu on pharmacokinetics of cefquinome following intramuscular administration (2 mg/kg body weight) in sheep was evaluated.

The high performance liquid chromatography apparatus comprising quaternary gradient delivery pump, UV detector, Autosampler and reverse phase C_{18} column at room temperature. The mobile phase consisted of water containing 0.1% Trifluroacetic acid as mobile phase A and Acetonitrile as mobile phase B in the gradient flow and pumped into column at a flow rate of 1.5 mL/min at ambient temperature. The HPLC data integration was performed using software Clarity (Version 2.4.0.190).

Following intramuscular administration of cefquinome in healthy sheep, peak plasma concentration of 4.36 ± 0.10 µg/mL observed at 0.75 h. Following intramuscular administration of cefquinome in tolfenamic acid - treated sheep, peak plasma concentration of 4.73 ± 0.05 µg/mL observed at 0.75 h was significantly higher than healthy sheep. The plasma drug concentrations were significantly higher from 0.083 to 18 h post administration of drug in tolfenamic acid - treated sheep compared to healthy sheep. Following intramuscular administration of cefquinome in healthy and tolfenamic acid treated sheep, the plasma drug concentration ≥ 0.15 µg/ml was detected up to 18 h. Following intramuscular administration of cefquinome in trikatu pretreated sheep, peak plasma concentration of 5.23 ± 0.08 µg/mL observed at 0.75 h was significantly higher than healthy sheep.

Following intramuscular administration of cefquinome in healthy sheep, $AUC_{(0-\infty)}$ was 16.65 ± 0.57 µg.h/mL, C_{max} was 4.36 ± 0.10 µg/mL and $Vd_{(area)}$ was 2.07 ±

0.34 L/kg. Following intramuscular administration of cefquinome in tolfenamic acid-treated sheep, values of K_a (2.75 ± 0.17 h^{-1}), α (0.36 ± 0.01 h^{-1}) and C_{max} (4.73 ± 0.05 μg/mL) were significantly higher and values of $t_{½}$ (1.95 ± 0.06 h) and $t_{½Ka}$ (0.26 ± 0.01 h) were significantly lower than the values obtained following intramuscular administration of cefquinome in healthy sheep. In trikatu pretreated sheep, pharmacokinetic parameters like distribution rate constant (α) 0.43 ± 0.03 h^{-1} and $AUC_{(0-\ )}$ (18.68 ± 0.14 μg.h/mL) were significantly higher in comparison to healthy sheep. Moreover, higher peak plasma concentration (C_{max} : 5.23 ± 0.08 μg/mL) was also observed in trikatu pre-treated sheep.

In safety study, daily intramuscular administration of cefquinome (2 mg/kg body weight) in combination with tolfenamic acid (2 mg/kg body weights) for 5 days was found safe with no significant alterations ($p < 0.05$) in all hematological and serum biochemical parameters in sheep.

The present study revealed that effect of tolfenamic acid administration altered pharmacokinetic profile of cefquinome following intramuscular administration in sheep. Therefore, concomitant use of cefquinome along with tolfenamic acid in sheep requires close therapeutic monitoring for potential pharmacokinetic drug interaction. Both cefquinome and tolfenamic acid were well tolerated following repeated intramuscular administration. However, alteration in therapeutic potential due to drug interactions needs to be evaluated in target species under field condition. Pretreatment of trikatu showed improvement in C_{max} and AUC studied. This has lead to cut down the frequency of administration and the related side effects. This may also bring down the cost of treatment of many infections. However, detailed intravenous study is envisaged.

Dr. A. M. Thaker
Professor and Head,
Department of Veterinary Pharmacology and Toxicology,
College of Veterinary Science and Animal Husbandry,
Anand Agricultural University,
Anand- 388 001

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON EFFECT OF TOLFENAMIC ACID AND BIOENHANCER TRIKATU ON PHARMACOKINETICS OF CEFQUINOME AND SAFETY OF SIMULTANEOUS ADMINISTRATION OF CEFQUINOME AND TOLFENAMIC ACID IN PATANWADI SHEEP"** submitted by **MAYANKKUMAR PRABHUBHAI RANA (Reg. No. 04-1724-2011)** in partial fulfillment of the requirements for the award of the degree of **MASTER OF VETERINARY SCIENCE in the subject of VETERINARY PHARMACOLOGY AND TOXICOLOGY,** of the Anand Agricultural University is record of bonafide research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Anand
Date: /02/2014

(A. M. Thaker)
Major Advisor

CERTIFICATE

This is to certify that, I have no objection for supplying to any scientist only one copy or any part of this thesis at a time through reprographic process, if necessary, for reference services in a library or documentation centre.

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Date: /02/2014

Mayankkumar P. Rana

Place: Anand
Date: /02/2014

(A. M. Thaker)

Major Advisor

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ABBREVIATIONS

α	Distribution rate constant
β	Elimination rate constant
<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
%	Per cent
µ	Micron
µl	Microliter
µg	Microgram
µg/ml	Microgram per milliliter
$\mu g \cdot h^2/ml$	Microgram square hour per milliliter
µg · h/ml	Microgram hour per milliliter
A	Zero-time intercept of distribution phase
AKP	Alkaline phosphatase
ALT (SGPT)	Alanine aminotranfserase
AST(SGOT)	Aspartate aminotranferase
AUC	Area under curve
AUMC	Area under first moment of curve
B	Zero-time intercept of elimination phase
BUN	Blood urea nitrogen
b.wt.	Body weight
$Cl_{(B)}$	Total body clearance
cmm	Cubic millimeter
C_{max}	Maximum drug concentration
Cp	Plasma concentration
C.V.	Coefficient of variance

et al.	et alibi
g	Gram
g/dl or gm/dl	Gram per desiliter
Hb	Haemoglobin
HPLC	High Performance Liquid Chromatography
IU	International unit
IU/L	International unit per liter
K_a	Absorption rate constant
kg	Kilogram
L or l	Liter
LDH	Lactate dehydrogenase
L/h/kg	Liter per hour per kilogram
L/kg	Liter per kilogram
mg	Milligram
mg/kg	Milligram per kilogram
mg/L	Milligram per liter
mg/dL	Milligram per desiliter
MIC	Minimum inhibitory concentration
Min	Minute
mL	Milliliter
MRT	Mean residence time
ng/g	Nenogram per gram
PCV	Packed cell volume
pH	Hydrogen ion concentration
rpm	Revolution per miniute
S.E.	Standard error
T	Time
$t_{½ K(a)}$	Absorption half life

$t_{1/2\alpha}$	Distribution half life
$t_{1/2\beta}$	Elimination half life
TEC	Total erythrocyte count
t_{max}	Time of maximum observed concentration in serum
$Vd_{(area)}$	Apparent volume of distribution
$Vd_{(ss)}$	Volume of distribution at steady state
v/v	Volume in volume
WBC	White blood cells

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CHAPTER - I

INTRODUCTION

Antimicrobial agents build up a major group of drugs extensively used in human and veterinary medicine to treat diseases caused by bacterial infection. Amongst beta-lactam antibiotics cephalosporins are well-known and very useful class of antibacterials, extensively used in veterinary medicine for preventing and treating of wide variety of bacterial infections (Becker *et al*., 2004). The first true representative of this class of antibiotics, cephalosporin C was discovered in culture broth of the fungus *Cephalosporium acremonium* in 1948. Development of 7 - aminocephalosporinic acid (7-ACA) by removing side chains of cephalosporin C became a template for chemists to produce many cephalosporins.

Cephalosporins are classified as first, second, third, fourth and fifth generation based on their general features of antimicrobial spectrum and chronology. Cephalosporins produce their bactericidal effect by inhibition of bacterial cell wall synthesis. Cephalosporins like other beta lactam antibiotics show “time dependent killing dynamics” (Sharma *et al.,* 2002).

Cefquinome is the first animal dedicated fourth generation cephalosporin antibiotics having broad spectrum of activity. It is a semi-synthetic, fourth-generation cephalosporin antibiotic effective against a wide range of bacteria and is highly resistant to -lactamase. The *in vitro* and *in vivo* efficacy of this drug is against a wide range of *gram-negative and gram-positive* bacterial pathogens and many anaerobic bacteria. It is resistant to the actions of -lactamases which inactivate many other cephalosporins, including ceftizoxime and ceftazidime. Cefquinome is active against many strains of *methicillin-resistant staphylococci* (MRSA) and *enterococci* (Limbert

et al., 1991; Murphy *et al*., 1994; Shpigel *et al*., 1997; Guerin-Faublee *et al*., 2003; Uney *et al*., 2011).

Cefquinome is relatively safer antibacterial having fewer adverse effects than other cephalosporins. Owing to its high efficacy, broad spectrum of activity, rapid tissue penetration, high safety and very low development of bacterial resistance, cefquinome is gaining popularity among the practitioners.

Sheep is considered as minor species of animals which is reared for wool and mutton production. Pneumonia and other bacterial infections in sheep are conditions which require therapy and antimicrobial drugs are likely to be used alone and in combination with non-steroidal anti-inflammatory drugs (NSAIDs). However, there is a limited literature on the pharmacokinetics of cefquinome, yet such data are essential as a basis for designing dosage schedules for clinical use in sheep.

Co-administration of several drugs often results in unpredictable therapeutic outcome. Often it either diminish therapeutic efficacy or increase toxicity of one or more of the administered drugs. NSAIDs are frequently recommended as an adjunct therapy with antibacterial to treat various bacterial infections accompanied by many inflammatory conditions in animals. Antimicrobial agents and NSAIDs are often concomitantly used to treat bacterial diseases like endotoxaemia, pneumonia and other bacterial diseases in sheep. Since there are reports of drug interactions among the NSAIDs and cephalosporin compounds (Singh *et al.,* 2013), it was thought worthwhile to study the effect of NSAIDs (tolfenamic acid) on the pharmacokinetics of cefquinome and to assess the safety and/or potential toxicity.

Tolfenamic acid (TA) is a NSAID of the fenamate sub-group. Chemically it resembles mefenamic acid and flufenamic acid, other fenamates in clinical use. Tolfenamic acid inhibits the biosynthesis of prostaglandins and also has inhibitory

action on prostaglandin receptors. The mechanism of action is by inhibition of cyclo-oxygenase (COX) enzyme (Linden *et al.,* 1975, 1976; McKellar *et al.,* 1994; Landoni and Lees, 1995, Landoni *et al.,* 1996[a,b]; Lees *et al.,* 1998) which, catalyses the conversion of arachidonic acid to pro-inflammatory prostaglandins (Vane, 1971). It has dual inhibitory action of COX and 5-lipoxygenase (5-LOX) with anti-oxidant properties at molecular level. Tolfenamic acid may be classified as an essentially non-selective inhibitor of the COX isoforms, COX-1 and COX-2, because there is 95% inhibition of PGE2 in exudates and 59% inhibition of TxB2 in serum (Lees, 2003)

Tolfenamic acid is used clinically as an anti-inflammatory and analgesic agent for the therapy of locomotor diseases in the dog (Robertson and Taylor, 2004) and febrile syndromes and post-operative pain in cats (Slingsby and Waterman-Pearson, 2000). Its anti-inflammatory and anti-endotoxaemic properties may also be used to enhance the rate of recovery in combination with antimicrobial drugs in acute mastitis in cattle, in pneumonia and other viral and bacterial respiratory diseases in calves and in pigs for the treatment of the metritis - mastitis -agalactia syndrome.

Plants and plant based derivatives are important part of the health care system since ancient human civilization. Recent advancement in bioavailability enhancement of drugs by compounds of herbal origin has created a revolutionary shift in the way of therapeutics. In ayurveda, the concept of bioenhancer is being used since centuries and is called “Yogvahi” (Annamalai and Manavalan, 1990; Johri and Zutshi, 1992). They are being prescribed routinely for a variety of diseases as part of multidrug formulations (Raj and Nagarsheth, 1978). Uses of bioenhancers are also applicable in veterinary practice since bioavailability of drugs and nutrients is of equal relevance to animals as to humans (Singh *et al*., 2009). Herbal bioenhancers have been shown to enhance bioavailability and bioefficacy of different classes of drugs, such as

antibiotics, antituberculosis, antiviral, antifungal and anticancerous drugs at low doses.In Ayurveda, Black Pepper (*Piper nigrum* Linn.), Long Pepper (*Piper longum* Linn.) and Ginger (*Zingiber officinalis*) collectively when combined in 1:1:1 proportion respectively is known as trikatu (Abd El-Aty and Goudah, 2002).

Piperine (1-piperoyl piperdine) is a major component of the Piper species. Piperine has various pharmacological activities namely inhibition of hepatic monooxygenase and uridine diphosphate (UDP) – glucuronyl transferase and intestinal glucuronidation (Atal *et. al*., 1981; Atal *et. al*., 1985; Singh *et. al*., 1986), inhibition of CYP3A4 and P-glycoprotein (PGP) (Bhardwaj *et. al*., 2002). In addition, Piperine has also been shown to enhance the bioavailability of various antibiotics like oxytetracycline, norfloxacin, ampicillin, ciprofloxacin, gatifloxacin, levofloxacin and -lactam antibiotics (Bhise and Pore, 2002; Hiwale *et. al*., 2002; Singh *et al*., 2005; Janakiraman and Manavalan, 2008[ab], Patel *et. al.,* 2011[b], Patel, 2012) and other drugs like vascicine, sparteine, curcumin, barbiturate, oxyphenylbutazone, zoxazolamine, propranalol and theophylline in animal experiments (Atal *et al*., 1981; Majumdar *et al*., 1990[ab]; Bano *et al*., 1991; Shobha *et al*.,1998; Majumdar *et al*., 1999). Looking to its bioenhancing property, trikatu can be applied for increasing bioavailability of cefquinome in sheep.

Data on pharmacokinetic interaction of cefquinome and tolfenamic acid, effect of trikatu on pharmacokinetics of cefquinome as well as safety of repeated administration of cefquinome in combination with tolfenamic acid following intramuscular (IM) administration have not been reported. Looking to this fact, the present study was therefore, undertaken to determine the effect of tolfenamic acid and bioenhancer trikatu on pharmacokinetics of cefquinome and safety of cefquinome in combination with tolfenamic acid in sheep.

OBJECTIVES

Studies on pharmacokinetic interaction and safety of cefquinome were undertaken in sheep with the following objectives.

A) To optimize and standardize the method for detection of cefquinome in plasma of sheep by High Performance Liquid Chromatography (HPLC).

B) To study pharmacokinetics of single dose IM administration of cefquinome (2 mg/kg) in healthy sheep.

C) To study the effect of IM administration of tolfenamic acid (2 mg/kg) on pharmacokinetics of single dose IM administration of cefquinome (2 mg/kg) in sheep.

D) To evaluate bioenhancing effect of trikatu (2 g/kg, PO) on pharmacokinetics of single dose IM administration of cefquinome (2 mg/kg) in sheep.

E) To evaluate safety of cefquinome (2 mg/kg) following multiple IM administration (2 mg/kg repeated at 24 hour intervals for 5 days) in combination with IM administration of tolfenamic acid (2 mg/kg) repeated at 24 hour intervals for 5 days in sheep.

CHAPTER - II

REVIEW OF LITERATURE

Cephalosporins are important class of antimicrobial agents in use today for both human and animals. Cephalosporins were discovered long back in 1948, but it was only in 1955 that active nucleus, cephalosporin C was isolated. In the year 1961 its chemical structure was identified at Oxford University and since onwards it has become a major group of clinically useful class of antibacterial drugs for treatment of a variety of infections caused by Gram-negative and Gram-positive bacteria. The spectrum of activity has been gradually widened from first-generation to fifth-generation cephalosporins. They are gaining importance as they are well tolerated and produce fewer side effects as compared to other classes of antibacterial drugs. The present review has been made to focus on various aspects of cefquinome, a fourth generation cephalosporin.

2.1 History and Source

Cephalosporins were first isolated from a fungus *Cephalosporium acremonium* by Brotzu in 1948 from the sea near a sewer outlet off Sardinian coast. Crude filtrate from the culture of this fungus was found to inhibit the *in-vitro* growth of *Staphylococcus aureus*. In addition it was found to be resistant to degradation by -lactamases. Crude filtrate contained three distinct antibiotics, which were named as cephalosporin P, cephalosporin N and cephalosporin C. The most active was cephalosporin C having the nucleus 7-aminocephalosporanic nucleus. Cephalosporins present ring structures that were found to be easily modified and so different compound were then synthesized leading to development of various classes of cephalosporins. With the addition of side chain in active nucleus, it became possible to produce semi-synthetic compounds with antibacterial activity much greater than

that of parent substance (Mandell and Petri, 1996). All semi-synthetics cephalosporins are derived from cephalosporin C. Cephalosporins antibiotics are a well-tolerated member of antibiotics in human and animals (Preston, 1992). Fourth-generation cephalosporins show marked resistance to -lactamases and increased outer membrane permeability, when compared with third-generation cephalosporins (Hancock and Bellido, 1992).

2.2 Chemistry and Structure activity relationship

Cephalosporins as well as penicillins are called beta-lactam antibiotics and are characterized by three fundamental structural requirements: the fused beta-lactam structure, a free carboxyl acid group, and one or more substituted amino acid side chains. The basic 7-aminocephalosporanic acid nucleus of cephalosporin contains a six-membered dihydrothiazine ring fused with a four membered -lactam ring (Figure 2.1). Penicillins have instead a five-membered (thiazolidine) ring attached to -lactam ring, 6-aminopenicillanic acid nucleus. In both the cases it is the -lactam ring that is essential for their antibacterial activity. In cephalosporins, the dihydrothiazine ring confers advantage to the –lactam ring in the terms of increased resistance to the action of extra chromosomally mediated staphylococcal -lactamases (penicillins) such that the cephalosporins have an inherently broader spectrum of activity than that of penicillins.

Since the -lactam ring is essential for the biological activity of these compounds, cleavage at any point of this ring results in complete loss of antibacterial activity. Substitution of various groups at R7 and R3 of the cephalosporin nucleus has generated a wide variety of compounds with differences in the spectra of activity and in various properties such as oral availability, stability to hydrolysis by -lactamases,

protein binding affinities and various other chemical susceptibilities (Hornish and Kotarski, 2002).

All the marketed cephalosporins are semi-synthetic; the basic nucleus is isolated from fermentation broth and the chemical substitutions are done. These variations in structure impact the drugs ability to get Penicillin Binding Proteins (PBPs) through porins of Gram-negative bacterium and affect other aspects of their bactericidal action. Cephalosporins have the advantages of lactamases stability, good activity against target proteins (PBPs) and good ability to penetrate bacterial cell wall.

Cefquinome is chemically 1-[[(6R,7R)-7-[[(2Z)-(2-Amino-4-thiazolyl) (methoxyimino) acetyl] amino] - 2 - carboxy - 8 - oxo - 5 - thia-1- azabicyclo [4.2.0]oct-2-en-3-yl] methyl]-5, 6, 7, 8 – tetrahydro-quinolinium inner salt (Figure 2.2). Cefquinome, an aminothiazolyl cephalosporin is the first member of fourth-generation cephalosporin developed solely for veterinary use (Guerin-Faublee *et al.,* 2003, Thomas *et al.,* 2006). Chemical formula of cefquinome is $C_{23}H_{24}N_6O_5S_2$. It has a molecular weight of 528.6. Cefquinome is generally available as its sulfate salt. Chemically cefquinome sulphates1-[[(6R, 7R) - 7-[[(2Z) - 2-(2 – Amino - 4-thiazolyl) -2-(MethoxyiMino) acetyl] amino] - 2 - carboxy - 8 - oxo-5-thia-1-azabicyclo [4.2.0]oct-2-en-3-yl] Methyl]- 5, 6, 7, 8 –tetrahydro-quinolinium sulfate. The chemical structure of cefquinome sulphate is depicted in Figure 2.3. The chemical formula of cefquinome sulfate is $C_{23}H_{24}N_6O_5S_2 \cdot H_2SO_4$. It has a molecular weight of 626.68.

Cefquinome and other fourth generation cephalosporins have a quaternary nitrogen that is positively charged at the 3-position, providing the properties of a zwitterions (Naito *et al.*, 1986; Rolinson, 1986). The chemical modifications of the

basic cephalosporin structure provide zwitterionic property to cefquinome. This property of cefquinome can facilitate rapid penetration across biological membranes, including the porins of the bacterial cell wall, enhance bioavailability and improve the spectrum of antimicrobial activity compared with the second and third generation cephalosporins (Sader and Jones,1993; Shpigel *et al.,* 1997; Guerin-Faublee *et al.,* 2003; Thomas *et al.,* 2006). Cefquinome is stable against chromosomally and plasmid-encoded lactamases that are produced by a majority of clinically important bacteria (Limbert *et al*., 1991). It has been approved for treatment of respiratory tract diseases, acute mastitis and foot rot in cattle, calf septicaemia, respiratory diseases in pigs, metritis-mastitis-agalactia syndrome in sows, foal septicaemia and respiratory tract diseases in horses. Potentially it will also be of therapeutic value in many sheep diseases. However, there are no published data on the clinical efficacy and the pharmacokinetics of cefquinome in sheep.

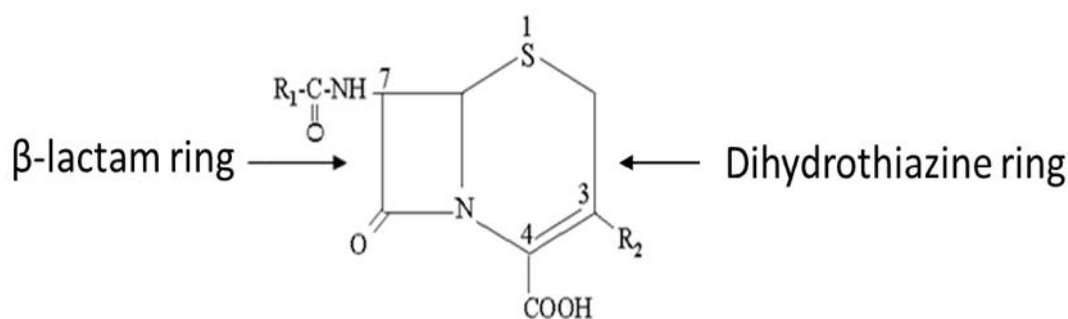

Figure 2.1: The general structure of Cephalosporins

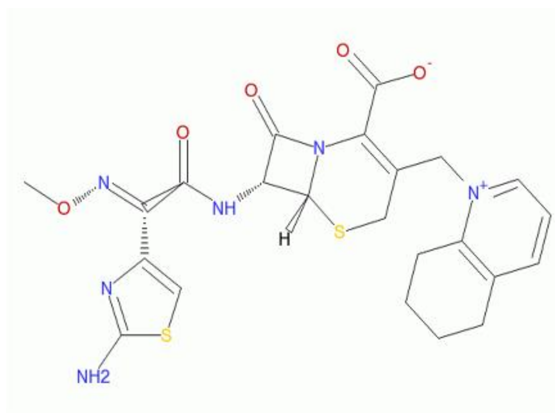

Figure 2.2: The chemical structure of cefquinome

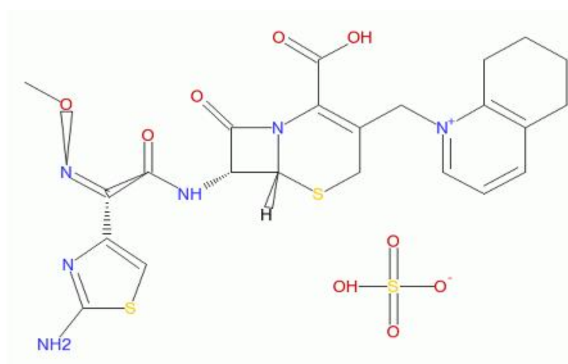

Figure 2.3: The chemical structure of cefquinome sulphate

2.3 Physical Properties

Cefquinome, a new semi synthetic aminothiazolyl cephalosporin is a broad spectrum beta-lactam classified as a fourth-generation cephalosporin (4GC) (Bryskier, 1997). It is available as sterile cefquinome sulphate suspension for parenteral use.

The Cefquinome sulphate is white or light yellow powder that is freely soluble in water. Freshly prepared solution of cefquinome sulphate displays clear transparent colour. The pH of the reconstituted solution ranges from 5 to 8.

2.4 Mechanism of Action

Cephalosporins produce their bactericidal effects by inhibition of bacterial cell wall synthesis and show "time-dependant killing dynamics"

(Sharma *et al*., 2002). The cell wall of bacteria is essential for their normal growth and development. Peptidoglycan is a heteropolymeric component of cell wall that provides rigid mechanical stability to cell wall. In Gram-positive microorganism, the cell wall is thick but thin in Gram-negative microorganisms. The peptidoglycan is composed of glycan chains, which are linear strands of two alternative amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) that are cross linked by peptide chains. The peptide varies, but begins with L-alanine and ends with D-alanine. In the middle is a dibasic amino acid, diaminopimelate (DAP). DAP provides a linkage to the D-alanine residue on an adjacent peptide (Salyers and Dixie, 2002).

Biosynthesis of peptidoglycan involves about thirty bacterial enzymes and may be considered in three stages (Mandell and Petri, 1996). In the first stage, precursor uridinediphosphate (UDP) - acetylmuramyl - pentapeptide, called a "Park nucleotide" is formed. A dipeptide, D-alanyl-D-alanine combines with this precursor. In the second stage, UDP-acetylmuramyl-pentapeptide and UDP-acetylglucosamine are linked with release of the uridine nucleotide to form a long polymer. The third and final stage of peptidoglycan synthesis is accomplished by transpeptidation reaction which involves linkage between the terminal glycine residue of pentaglycine bridge and fourth residue (D-alanine) of pentapeptide, releasing the fifth residue (D-alanine). The cross linking is catalysed by the enzyme transpeptidase. It is the last step in peptidoglycan synthesis that is inhibited by the -lactam antibiotics.

Cephalosporins bind at the active site of the transpeptidase enzyme that crosslinks the peptidoglycan strands. The -lactam ring is bound to it as the ring structurally imitates the D-alanine peptide of the peptidoglycan side chains would normally bind to this site. They inhibit the enzyme irreversibly by reacting with a serine residue in the transpeptidase, resulting in defective bacterial cell wall

(Caprile, 1988) and the resulting weakened bacterial cell wall eventually ruptures causing cell lysis (Thomson *et al*., 1984). Cephalosporins do not inhibit cell wall synthesis in mammals as they lack similar cell wall structure.

There are additional, related targets for the action of penicillins and cephalosporins termed as penicillin-binding proteins (PBPs). Cephalosporins inhibit bacterial cell wall synthesis by preferentially binding to the bacterial cell wall, resulting in inhibition of bacterial growth or bacterial cell lysis and death (Neu, 1982; Papich, 1984 and Hornish and Kotarski, 2002). Penicillin-binding proteins are enzymes involved in various stages of cell wall synthesis. They are found in quantities of several hundred to several thousand molecules per bacterial cell. PBPs vary among different bacterial species. Thus the intrinsic activity of cephalosporins against a particular organism depends on their ability to gain access to and bind the necessary PBPs (Papich, 1987).

Cefquinome is the only fourth generation cephalosporin that is developed especially for use in animals. Like other beta-lactams, cefquinome is bactericidal by inhibiting the cell wall synthesis of actively growing bacteria. The cefquinome molecule differs from third-generation cephalosporins (3GCs), e.g. cefotaxime, by a quarternary ammonium side chain attached to the C-3 position of the beta-lactam nucleus. As with other 4GCs, cefquinome is a zwitter-ionic compound with improved penetration into the periplasmatic space of Gram-negative bacilli and enhanced binding to penicillin-binding proteins. As a result of their molecular structure, 4GCs show a higher stability against beta-lactamase (Bryskier, 1997).

2.5 Classification of Cephalosporins

The explosive growth of the cephalosporins during the past decade has necessitated formulating a system of classification. The well-accepted system of

classification of cephalosporins, by generations or chronology is very useful. Each of the newer generations introduced has some added general advantages over the previous generation (Prescott and Baggot, 1993).

Clinically, the cephalosporins are classified as first-, second-, third- and fourth-generation, reflecting in part their relative in-vitro spectrum of activity (narrow, expanded, broad and extended, respectively), structural similarities, and to a certain extent the time of their introduction into the market (Caprile, 1988; Livermore and Williams, 1996, Chaudhary, 2003). Classification of cephalosporins along with general properties of each class has been presented in Table 2.1.

The first-generation cephalosporins are usually active against Gram-positive pathogenic cocci including penicillinase-producing and nonpenicillinase-producing *Staphylococcus aureus* and *S. epidermidis*; group A beta-hemolytic streptococci; and group B streptococci. In general, first-generation cephalosporins have very limited activity against Gram-negative bacteria.

The second-generation cephalosporins are generally active against the same strains of organisms that are susceptible to first-generation cephalosporins, but they have additional activity against various organisms such as *Haemophilus influenzae*. Second-generation cephalosporins are generally more active against Gram-negative bacteria than first generation ones, although the scope is not very large.

The third-generation cephalosporins were designed to have enhanced activity against Gram-negative bacteria, while retaining good activity for Gram-positive bacteria. They are usually less active against susceptible staphylococci than first-generation cephalosporins. Compounds in this class have enhanced hydrolytic stability to many of the -lactamases that are active against the earlier generation cephalosporins and penicillin (Hornish and Kotarski, 2002). The oxyimino side chains

Table 2.1: Classification of Cephalosporins*

GENERATIONS	AGENTS	GENERAL PROPERTIES
1st generation (Narrow-Spectrum)	Cefadroxil, Cefazolin, Cephalexin, Cephapirin, Cephradine, Cephalothin, Cephaloridine	• Highest activity against Gram-positive bacteria including most *Corynebacteria, Streptococci* and *Staphylococci* spp. • Moderate activity against gram-negative bacteria. • Orally active. • Susceptible to β-lactamases. • Do not cross the blood-brain barrier.
2nd generation (Expanded-Spectrum)	Cefaclor, Cefamandole, Cefmetazole, Cefonicid, Ceforanide, Cefotetan, Cefoxitin, Cefprozil, Cefurozime, Loracarbef	• More effective against Gram-negative bacteria and *Bacteroides fragilis*. • Less efficacy than first generation cephalosporins against Gram-positive pathogens. • Do not achieve adequate levels in the cerebrospinal fluid.
3rd generation (Broad-Spectrum)	Cefbuperazone, Cefixime, Cefoperazone, Cefotaxime, Cefpodoxime, Cefdinir Cefsulodin, Ceftazidime, Ceftiofur, Cefmenoxime, Ceftizoxime, Ceftriaxone, Moxalactam, Ceftibuten	• More active against many resistant Gram-negative organisms (some isolates of *P. aeruginosa*). • Excellent activity against *Enterobacteria*. • More resistant to non-staphylococcus - lactamase. • Long half-life. • Readily cross the blood-brain barrier.
4th generation (Extended-Spectrum)	Cefepime, Cefpirome, **Cefquinome**	• Stable to hydrolysis of β-lactamases. • Active against many Enterobacteria resistant to many other cephalosporins. • Excellent penetration into CSF and peritoneal fluid. • Broader Gram-positive activity. • Less activity against *Pseudomonas spp.* than third generation cephalosporins. • Zwitterionic property that can penetrate the outer membrane of Gram-negative bacteria
5th generation	Ceftobiprole,Ceftaroline	• Unique in its activity against Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Staphylococcus aureus.*

*Hornish and Kotarski, 2002; Kollef, 2009;Sweet and Gibbs 2009

present in most of the third-generation cephalosporins were introduced to confer stability of the -lactam ring to certain -lactamases (i.e., the TEM-1, TEM-2 and SHV-1 -lactamases) that emerged following the selective pressure exerted by use of ampicillin, carbenicillin, and the first- and second-generation cephalosporins in the 1960's (Livermore and Williams, 1996). The most of third generation cephalosporins also have some activity against *Pseudomonas* spp. Some of them show good cerebrospinal fluid penetration.

The fourth-generation cephalosporins have enhanced activity against the organisms susceptible to third-generation cephalosporins. They have an expanded spectrum of activity against Gram-negative bacteria compared to other classes of cephalosporins (Hornish and Kotarski, 2002).

Generally with introduction of newer generations there is an increased activity against various Gram-negative bacteria and decreased susceptibility to -lactamases.

2.6 Antimicrobial Spectrum

Cephalosporins possess broad-spectrum activity against a wide variety of microorganisms including those which are resistant to conventional antibiotics. In general, first generation cephalosporins are active against *Staphylococcus spp., Streptococcus spp., Escherichia coli*, *Proteus smirabilis* and *Klebsiellla spp.* but do not have activity against methicillin - resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), anaerobic bacteria, *Enterobacter spp., Enterococcus spp., Proteus spp. and Serratia spp*. Second generation cephalosporins are active against same bacteria as first generation cephalosporins and also have activity against some strains of *Enterobacter, Escherichia coli, Klebsiellla, Proteus* and *Serratia* that are resistant to first generation cephalosporins. Cefoxitin, cefotetan, cefmetazole and cefamandole have some activity

against anaerobic bacteria and cefuroxime has greater stability against β-lactamases. Cefaclor has more activity against gram-negative bacteria. Third generation cephalosporins have less activity against *Staphylococci* but greater activity against Gram-negative bacteria than other cephalosporins and also have activity against some strains of *Enterobacter, Escherichia coli, Klebsiellla, Proteus* and *Serratia* that are resistant to first and second generation cephalosporins. Most third generation cephalosporins have activity against *Pseudomonas spp.* Cefotaxime has better gram-positive and anaerobic activity whereas cefixime, ceftriaxone and ceftazidime have limited anaerobic activity and ceftizoxime has good anaerobic activity (Vaden and Riviere, 2001). Cephalothin and cefadroxil have greatest activity against *Staphylococci* (Papich, 1984); *Staphylococcus epidermidis* is only variably susceptible to cephalexin and cefadroxil (Caprile, 1988).

The fourth-generation cephalosporins have enhanced activity against the organisms susceptible to third-generation cephalosporins. They have an expanded spectrum of activity against Gram-negative bacteria compared to other classes of cephalosporins (Hornish and Kotarski, 2002).The fourth-generation cephalosporins are structurally related to the third-generation cephalosporins but, in addition, they possess a quaternary ammonium group at the C-3' position. They are zwitterionic compounds, which facilitates rapid penetration through the outer membrane of Gram-negative bacteria. This, together with their low affinity for clinically important -lactamases, results in potent activity against many Gram-negative pathogens, including strains producing derepressed class I (AmpC) -lactamase, resistant to most third-generation cephalosporins. In addition, some fourth generation cephalosporins exhibit excellent activity *in vitro* against Gram-positive bacteria, including methicillin

susceptible *staphylococci*, penicillin-resistant *pneumococci* and *viridans* group *streptococci* (Garau *et al*., 1997).

The fourth-generation cephalosporins, unlike third-generation cephalosporins, are active *in vitro* against Gram-negative bacilli which produce depressed amounts of AmpC -lactamases (Hancock and Bellido, 1996). Antimicrobial activity and MIC of cefquinome had been tested and compared with other cephalosporins by a number of workers (Thomas *et al.,* 2006; Bottner *et al*., 1995; Chin *et al*., 1992,)

In a review of the activity of third and fourth generation cephalosporins, cefquinome was extremely active against group-A *streptococci* and *Streptococcus pneumonia* (Chin *et al.,* 1992). Cefquinome is highly resistant to hydrolysis by plasmid encoded -lactamases from *Escherichia coli*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*, as well as by chromosomal-encoded lactamases from *Citrobacter* species, *Enterobacter cloacae* and *Klebsiella oxytoca*. In contrast to most of the third-generation cephalosporins, cefquinome is also active against many strains of methicillin-resistant *staphylococci* and *enterococci* (Limbert *et al.,* 1991).

Cefquinome was shown to have a broad spectrum of activity which covers many equine pathogens. (Thomas *et al.,* 2006). Cefquinome was very potent against fastidious isolates such as *Moraxella catarrhalis, Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Streptococcus* species. (Murphy *et al.,* 1994). Additionally, cefquinome has a good activity against causative agents of respiratory tract infections, diarrhoea and mastitis in cattle (Kikuchi *et al*., 1995; Wilson *et al*., 1996; Barkema *et al*., 1998; Shpigel and Schmid, 1997). Cefquinome is highly active against *P. multocida* and *E. coli* (Dinakaran *et al*., 2013).

Table 2.2: MICs (µg ml^{-1}) of cefquinome against pathogenic microbes*

Pathogen	**MIC50 (~g ml^{-1})**	**MIC90 (~g ml^{-1})**
Gram-positive organisms		
Clostridium perfringens	1	1
Staphylococcus aureus/intermedius	0.25	0.5
Streptococcus equi sub spp. *zooepidemicus*	0.016	0.032
Staphylococcus aureus (Methicillin-susceptible)	0.781	1.563
Staphylococcus aureus (Methicillin-resistant)	12.5	25
Streptococcus spp., serogroups A, B, and C	<0.006	0.024
Enterococci	4	32
Moraxella catarrhalis	-	0.25-2
Haemophilus influenzae	-	0.06-1
Neisseria gonorrhoeae	-	0.06-0.5
Gram-negative organisms		
Enterobacter spp.	*0.098*	*0.781*
Escherichia coli	0.063	0.125
Actinobacillus equi	0.008	0.016
Pseudomonas spp.	4	8
Pasteurella multocida	0.032	0.032
Manhemia haemolytica	0.032	0.063
Pseudomonas aeruginosa	6.25	25
Proteus spp. (indole positive)	0.195	25
Proteus mirabilis	0.098	0.195
Citrobacter spp.	0.049	0.195
Serratia marcescens	0.195	0.391
Klebsiella spp.	0.049	0.391
Salmonella spp.	0.098	0.195

*(Limbert *et al.,* 1991;Murphy *et al,* 1994; Thomas *et al.,*2006;Ehinger *et al.*, 2006;Zonca *et al.*, 2011).

2.7 Mechanism of bacterial resistance

Three independent factors determine the bacterial susceptibility to β-lactam antibiotics: production of β-lactamases, permeability of cell wall and sensitivity of penicillin binding proteins (Frere *et al.,* 1991). Bacterial resistance to cephalosporins may be related to inability to reach its sites of action and alterations in the penicillin binding proteins (PBPs) that are targets of cephalosporins (Petri, 2001).

For an antimicrobial agent to inhibit bacterial growth, it must be able to reach susceptible target site(s) in sufficient quantities. Bacteria, therefore, may resist the effects of antimicrobial agents by preventing their uptake, by inactivating them before they can act upon the target site(s) or by not having a target site that is susceptible to the effects of the antibiotic (Murray and Moellering, 1978). Beta-lactam antibiotics, for example, must reach the penicillin-binding proteins and the enzymes associated with peptidoglycan (murein layer) formation. Gram-negative bacteria have an outer phospholipid membrane surrounding the murein layer that can retard the entry of various substances, including β-lactam antibiotics (Leive, 1974). Alteration of the β-lactam target site(s) (the PBPs and the peptidoglycan enzymes) or variations in the affinity of various β-lactams for the target site(s) also lead to varied morphologic responses and even resistance to these antibiotics (Tomasz, 1979; Curtis *et al.,* 1979). Alterations in two PBPs (1A and 2X), such that they bind cephalosporins with lower affinity, are sufficient to render pneumococcal resistance to third generation cephalosporins, as the other three high molecular weight PBPs have inherently low affinity (Spratt, 1994).

The major mechanism by which bacteria resist β-lactam antibiotics are the production of β-1actamase(s), a group of enzymes that can hydrolyze the β-lactam ring and render the antibiotic inactive. The effectiveness of a β-lactamase in

conferring resistance upon the producing strains depends on an interaction of various factors, including affinity of the enzyme for the β-lactam antibiotics, velocity of the hydrolysis reaction and probably most importantly, the rate of delivery of the antibiotic to the enzyme, which in turn seems to be governed by the outer phospholipid membrane present in Gram-negative organisms (Murray and Moellering, 1978). There are two mechanisms of β-lactamase production: chromosomal and plasmid. Chromosomally derived β-lactamases is species and genus specific and induced by the presence of any β-lactam compound. Plasmid derived β-lactamases can be transferred between bacteria, increasing the resistance (Graham *et al.,* 1992). *Enterobacter spp.* have derived resistance to aminopenicillins and third generation cephalosporins, sometimes after single antibiotic injection due to their chromosomally mediated -lactamase (Marchou *et al.,* 1987), which make inactive all currently available -lactams, with the exception of fourth-generation cephalosporins (Mimoz *et al.,* 1997).

Gram-positive bacteria generally produce chromosomally derived β-lactamases. The chromosomally derived β-lactamases produced by Gram-negative bacteria are primarily cephalosporinases while the plasmid mediated ones are generally broader spectrum (Caprile, 1988; Sanders and Sanders, 1988). Gram-negative bacteria secrete small amounts of β-lactamases into their periplasmic space, allowing for optimal location of enzyme to degrade cephalosporins upon entry into organism. Cefoxitin and cefotetan are stable to chromosomally mediate β-lactamases, which give them excellent activity against anaerobic gram-negative rods (Vaden and Riviere, 2001). Cefazolin is susceptible to β-lactamases from *Staphylococcus aureus* than the cephalothin. Third generation cephalosporins are resistant to β-lactamases

produced by Gram-negative bacteria than first generation cephalosporins. Third generation cephalosporins are susceptible to inducible, chromosomally encoded (type 1) β-lactamases produced by aerobic gram-negative bacilli but fourth generation cephalosporins are poor inducers and less susceptible to hydrolysis by type 1 β-lactamases than the third generation agents (Petri, 2001).

Extended spectrum -lactamases (ESBL) are plasmid mediated and are mutants of older broad-spectrum -lactamases TEM-1, TEM-2 and SHV-1 (Caprile, 1988; Sanders and Sanders, 1988 and Mimoz *et al.,* 1999). The organisms most commonly associated with ESBL production are *Escherichia coli* and *Klebsiella pneumonie* (Barradel and Bryson, 1994).

Fourth-generation cephalosporins are poor inducers and less susceptible to hydrolysis by type 1 -lactamases than the third-generation cephalosporins (Petri, 2001). Cefquinome was not destroyed by the common plasmid -lactamases TEM-1, TEM-2, SHV-1, or by the chromosomal -lactamases of *Klebsiella, Branhamella* and *Pseudomonas*. Its activity was not adversely decreased in different medium or protein, and minimum bactericidal concentrations (MBCs) for most species except for *Enterobacter* were within a dilution of MICs (Chin *et al*., 1992).

The potential for fourth-generation cephalosporins to induce AmpC-type mechanisms of resistance is lower than for other beta-lactams (Jones, 1998). Also, fourth-generation cephalosporins are less likely to be hydrolyzed by extended spectrum beta-lactamases (ESBLs) than third-generation cephalosporins (e.g. ceftriaxone) (Bryskier, 1997). Resistance development to 4GCs is a multi-step process with a change in outer membrane proteins (decreased permeability) and the presence of extended beta-lactamase activity.

A change in outer membrane proteins (OMP) is a different mechanism of resistance in comparison to enzymatic hydrolysis via beta-lactamases, such as AmpC and ESBLs. Membrane protein changes can result in decreased permeability of the antimicrobial or increased efflux preventing the compound interacting with intracellular target molecules (PBPs). OMP resistance is chromosomally encoded and based on the occurrence of a mutational event and therefore not among the transferable mechanisms. Multi-passage studies with *Enterobacter cloacae* variants showed that resistant strains selected by fourth-generation cephalosporins differ from those selected by third-generation cephalosporins. Eighty percent (vs. 10% for by third-generation cephalosporins) of strains selected by fourth-generation cephalosporins lacked or had diminished levels of a 39 to 40 kDa major porin protein known to be involved in the permeation of cephalosporins (Fung-Tomc *et al*., 1989).

Beta-lactamases producing strains of *Enterobacteriaceae*, *Escherichia coli* and *Pseudomonas aeruginosa* that confer resistance to ceftazidime and cefotaxime have less effect on cefepime (Okamoto *et al.,* 1994). Alteration in porin channels in several strains of *Escherichia coli* resulted in variable activity of cefepime but the organisms still remained susceptible to cefepime at MIC of 8 µg ml^{-1}. Alteration in porin channels had relatively little effect on -lactamases-mediated resistance (Jacoby and Sutton, 1985). Various strains of *Escherichia coli* with different -lactamase types show low level of resistance to cefepime. Some strains of *Escherichia coli* that produce large amount of CEP-1 chromosome-mediated -lactamase remain susceptible to cefepime at MIC $\leq$ 0.25 µg ml^{-1} but became resistant to cefotaxime, ceftazidime, cefoxitin and aztreonam.

Resistance and cross-resistance to extended spectrum cephalosporins are reported to be rare among *Pseudomonas spp.* which may require multiple mutations

(Fung-Tomc *et al.,* 1989). The *in vitro* and *in vivo* efficacy testing showed that a wide range of gram-negative and gram-positive bacterial pathogens are susceptible to cefquinome. (Limbert *et al.* 1991). Additionally, cefquinome has shown a potent *in vitro* activity against gram-positive and gram-negative bacteria isolated from pigs and calves (Murphy *et al*., 1994; Bottner *et al*., 1995).

2.8 General pharmacokinetics

Pharmacokinetics deals with the study of rate of absorption, distribution, metabolism and excretion of drugs. It is described as the mathematical description of temporal changes in the concentration of drugs within body (Baggot, 1977). It is mainly concerned with the passage of drug to its site of action and maintenance of adequate concentration to exert its therapeutic, diagnostic or toxicological effect. The pharmacokinetic study of drugs provides an important tool to achieve the effective dosage regimen.

2.9 Pharmacokinetics of cefquinome

Cefquinome exhibits dose linear pharmacokinetic behaviour (Limbert *et al.,* 1991, Yuan *et al.,* 2011). It shows rapid and complete absorption following intramuscular administration (Uney *et al*., 2011). It is widely distributed throughout body tissues and fluid including bronchial mucosal tissues, peritoneal fluid, biliary fluid and milk (Ehinger *et al*., 2006,). Cefquinome shows excellent penetration in cows with experimentally induced mastitis using *Escherichia coli*. (Shpigel *et al.,* 1997). Important pharmacokinetic cefquinome in various species of animals have been depicted in Table 2.3.

2.9.1 Piglets

Song *et al.* (2013) studied influence of the injection site on the pharmacokinetics of cefquinome following intramuscular injection in piglets at dose

rate of 2 mg/kg body weight. The mean maximum concentrations (C_{max}) of cefquinome following intramuscular (IM) injection into neck or thigh area were 4.62 ± 0.31 µg/ml at 0.38 ± 0.14 hr and 4.39 ± 0.53 µg/ml at 0.42 ± 0.13 hr respectively. The elimination half-lives of cefquinome following IM injection into neck or thigh area were 1.57 ± 0.23, 1.56 ± 0.02 hr, respectively. The absolute bioavailability (F) of cefquinome after IM injection into the neck or thigh area were 103.04 ± 13.01 and 97.56 ± 16.14%, respectively ($P>0.05$). There were no differences noted between the two different injection sites for the pharmacokinetic properties of cefquinome after IM injection in piglets.

2.9.2 Chickens

Xie *et al.* (2013) studied the pharmacokinetics of cefquinome in chickens following IM and intravenous (IV) administration at dose rate of 2 mg/kg. Following IV injection distribution half-life was 0.43 ± 0.19 h, elimination half-life was 1.29 ± 0.10 h, total body clearance 0.35 ± 0.04 l/kg/h, area under curve was 5.33 ± 0.55 mg/h/ml and volume of distribution at steady state was 0.49 ± 0.05 l/kg. Following IM administration absorption half-life was 0.070 ± 0.02 h, distribution half-life was 0.58 ± 0.27 h, elimination half-life was 1.35 ± 0.20 h, peak concentration was 3.04 ± 0.71 mg/ml and bioavailability was 95.81 ± 5.81%.

2.9.3 Buffalo calves

Dinakaran *et al.* (2013) studied the pharmacokinetics of cefquinome in buffalo calves at dose rate of 2 mg/kg. Following IV injection distribution area under curve, elimination half-life ($t_{½}$), total body clearance (Cl_B) and mean residence time were 32.9 ± 0.56 µg·h/mL, 3.56 ± 0.05 h, 60.9 ± 1.09 mL/h/kg, and 4.24 ± 0.09 h, respectively.

2.9.4 Goats

Dumka *et al.* (2013) studied the pharmacokinetics of cefquinome in goats following single IV and IM administration at dose rate of 2 mg/kg. Following IV injection the elimination half-life ($t_{½}$) was 5.76 ± 0.19 h, $V_{d(area)}$ was 0.51 ± 0.05 L/kg, AUC was 33.83 ± 2.53 μg/mL, Cl_B was 0.06 ± 0.004 L/h/kg and MRT was 6.09 ± 0.11 h. Following IM administration, C_{max} was 4.84 ± 0.23 μg/mL, T_{max} was 1.5 h, elimination half-life ($t_{½}$) was 5.86 ± 0.29 h, AUC was 19.82 ± 2.07 μg·h/mL, MRT was 8.08 ± 0.50 h and bioavailability was 57.39 ± 3.40%.

2.9.5 New Forest Ponies

Smiet *et al.* (2012) studied the pharmacokinetics of cefquinome in new forest ponies following intravenous (IV) administration at dose rate of 1 mg/kg q12h, 1 mg/kg body weight q8h or 4.5 mg/kg body weight twice a day to each age group (n=6). Both Cl_B and MRT decreased as the age of the foals increased. Values of AUC increased, in a dose dependent manner, with significant increases for all age groups following administration of 4.5 mg/kg body weight q12h. Total body clearance did not have comparable dose dependency.

2.9.6 Sheep

Tohamy (2011) studied age-related pharmacokinetics of cefquinome in sheep following a single intramuscular (IM) administration at the doses of 1 and 10 mg/kg. Following IM administration of cefquinome, the absorption half-lives [$t_{1/2(ab)}$] were 1.540, 1.037 and 0.664 h at a dose of 1 mg/kg body weight and 1.844, 1.290 and 1.605 h at a dose of 10 mg/kg body weight in one, six-months and one year old sheep, respectively. After the two doses, C_{max} of 0.732, 1.145, 1.205 and 3.525, 5.088, 4.576 μg/ml were attained in the three ages, respectively. The $t_{1/2}$ and MRT values of cefquinome were longer in one-month old sheep compared to six-month old and

yearling sheep. The absorption and elimination processes were delayed in new born sheep of one-month old in contrary to six-month and yearling animals.

2.9.7 Ducks

Yuan *et al.* (2011) studied pharmacokinetics of cefquinome in healthy ducks following IV, IM and oral administration at dose rate of 5 mg/kg body weight. After IV administration, $t_{1/2}$ was 1.57 ± 0.06 h, Cl_B was 0.22 ± 0.02 l/kg·h and Vd(area) at steady state was 0.41 ± 0.04 L/kg. After IM administration, $t_{1/2}$ was 1.79 ± 0.13 hours, C_{max} was 9.38 ± 1.61 μg/mL and bioavailability was 93.28 ± 13.89 %. No cefquinome was detected in plasma after oral administration.

2.9.8 Camels

Al-Taher (2010) studied pharmacokinetics of cefquinome in camels following single IM administration of 1 mg/kg body weight. After IM administration, C_{max} was found to be 1.23 μg/mL. The $t_{1/2}$ was 10.24 h. The Mean Residence Time (MRT) was 16.74 h and AUC_{0-} was 20.37 μg/mL/h.

2.9.9 Pigs

Yang *et al.* (2009) studied pharmacokinetics of cefquinome in pigs following IV and IM administration at dose rate of 1 mg/kg body weight. After IV administration, $t_{1/2}$ was 1.34 h, $V_{d(area)}$ was 0.24 l/kg, Cl_B was 0.26 l/kg/h and AUC was 3.97 mg/l/h. After IM administration, $t_{1/2}$ was 2.76 h, C_{max} was 1.80 mg/l, Cl_B was 0.25 l/kg/h, AUC was 4.12 mg/l/h and F was 102.37%.

2.9.10 Piglets

Li *et al.* (2008) studied pharmacokinetics of cefquinome in healthy piglets following IV and IM administration at dose rate of 2.0 mg/kg of body weight. After IV administration, elimination half-life ($t_{1/2}$) was 1.85 ± 1.11 h, total body clearance (Cl_B) was 0.26 ± 0.08 l/kg·h, AUC was 8.07 ± 1.91 μg·h/mL and volume of

distribution at steady state (V_{SS}) was 0.46 ± 0.10 L/kg. After IM administration, $t_{1/2}$ was 4.36 ± 2.35 h, peak concentration (C_{max}) was 4.01 ± 0.57 µg/mL and bioavailability (F) was 95.13 ± 9.93%.

2.9.11 Buffalo Calves, Cattle Calves, Cows and Goats

Tohamy *et al.,* (2006) studied comparative pharmacokinetics of cefquinome in buffalo calves, cattle calves, cows and goats following single IM administration at dose rate of 1 mg/kg body weight. After IM administration the C_{max} were 0.659, 0.766, 0.587 and 0.603 µg ml^{-1} achieved after maximum time t_{max} of 7.53, 4.60, 4.079 and 9.230 hour post-injection in buffalo and cattle calves and lactating cows and goats, respectively. The absorption half lives ($t_{½(ab)}$) were 2.359, 1.017, 1.124 and 4.45 h and the elimination half-lives ($t_{½(el)}$) were 12.86, 13.46, 7.10 and 8.68 h for buffalo calves, cattle calves, lactating cattle and lactating goats, respectively and AUC were 17.76 ± 1.97, 18.67 ± 1.07, 8.92 ± 0.53 and 14.36 ± 1.2 µg ml^{-1} h^{-1} for buffalo calves, cattle calves, lactating cattle and lactating goats, respectively.

2.9.12 Mice, Dogs, Pigs and Calves

Limbert *et al.* (1991) studied pharmacokinetic of cefquinome in mice, dogs, pigs and calves following single IM administration at dose rate of 10 mg/kg body weight. After SC administration in mice, the C_{max} was 7.5 ± 2.9 µg ml^{-1} achieved after maximum time T_{max} of 0.38 ± 0.16 hour post-injection. After IV administration in dogs, $t_{1/2}$ was 0.98 ± 0.28 h, $Vss_{(area)}$ was 0.24 ± 0.09 l/kg and AUC was 50.4 ± 13.7 mg · h/l. After IV administration in pigs, $t_{1/2}$ was 1.32 ± 0.18 h, $Vss_{(area)}$ was 0.24 ± 0.11 l/kg and AUC was 66.7 ± 27.2 mg · h /l. After IV administration in calves, $t_{1/2}$ was 1.33 ± 0.41 h, $Vss_{(area)}$ was 0.23 ± 0.13 l/kg and AUC was 84.7 ± 25.3 mg · h /l.

Table 2.3: Pharmacokinetics of Cefquinome in animals

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2.10 Interaction of Cephalosporins with NSAIDs

Drug-drug interactions can be categorized into those originating from pharmacokinetic mechanisms and those originating from pharmacodynamics mechanisms. Pharmacokinetic interactions are those that result in alterations of drug absorption, distribution, metabolism and elimination; pharmacodynamics interactions occur when one drug affects the actions of another drug. The potential for drug-drug interactions is an important aspect of overall drug safety. Newer cephalosporins antimicrobials have been widely used in the clinical field because of their high clinical efficacy and safety and are often used concomitantly with other drugs.

There are number of reported drug interactions of cephalosporins with essential and trace elements (Domenico *et al.,* 1992; Thomas and Burns 1998; Ahmed *et al.,* 2000; Arayne *et al.,* 2001; Arayne *et al*., 2006; Sultana *et al.,* 2003; Sultana *et al.,* 2005; Jimoh *et al.,* 2011; Singh *et al.,* 2008; Patel[a] *et al*., 2012; Patel[b] *et al*., 2012).

Carbon *et al.* (1981) studied the effects of intramuscular administration of phenylbutazone (10 mg/kg) on the pharmacokinetics of cefazolin (30 mg/kg) administered intravenously in rabbits. When co-administered with phenylbutazone, significant increase in the value of area under curve (AUC) from 46.42 ± 4.37 to 75.42 ± 2.03 µg h^{-1} ml^{-1} and significant decrease in total body clearance from 11.33 ± 1.08 to 6.64 ± 1.78 ml min^{-1} was observed. No significant alteration in the values of elimination rate constant ( ) and elimination half life ($t_{1/2}$) was observed. They also studied effect of intramuscular administration of phenylbutazone (10 or 100 mg/kg injected simultaneously or 10 mg/kg injected 4 h before cefazolin injection) on cefazolin (30 mg/kg) administered intramuscularly in rabbits. Significant increase in

the $t_{1/2}$ of cefazolin in presence of phenylbutazone compared to the control was observed.

Joly *et al.* (1988) studied the effect of diclofenac co-administration on the kinetics of cefotiam, cefmenoxime and ceftriaxone in rabbits and compared the antibacterial effect of these antibiotics, given alone or with diclofenac, in experimental endocarditis. Diclofenac significantly increased the area under the curve of ceftriaxone (326.0 ± 91.4 vs 555.0 ± 124.0 µg h^{-1} mL^{-1}) and cefotiam (17.5 ± 4.4 vs 32.9 ± 17.9 µg h^{-1} mL^{-1}) and the terminal half-life (2.8 ± 0.5 vs 3.45 ± 0.4 h) of ceftriaxone. When cefmenoxime was given alone and co-administered with diclofenac, AUC in serum was 22.0 ± 9.9 and 21.7 ± 11.1 µg h^{-1} mL^{-1} respectively. Diclofenac reduced the renal clearance of cefotiam from 23.7 ± 6 to 17 ± 5 ml $min^{-1}kg^{-1}$ while that of cefmenoxime and ceftriaxone remain unchanged. No significant alteration in the value of elimination of half life ($t_{1/2}$) was observed after administration of cefotiam and cefmenoxime alone or co-administered with diclofenac.

The pharmacokinetic interaction of ceftizoxime (10 mg/kg, intramuscular) with paracetamol (50 mg/kg, intramuscular) was investigated in crossbred calves by Singh *et al.* (2008). Following administration of ceftizoxime alone, the peak plasma level (C_{max}: 24.9 ± 1.11 µg ml^{-1}) was attained at t_{max} of 45 min and the drug was detected in plasma above the minimum therapeutic concentration for up to 6 h post-administration. The disposition pattern of ceftizoxime followed one-compartment open model. The values of absorption half-life (t_{Ka}), elimination half-life ($t_{1/2}$) and AUC were 0.23 ± 0.03 h, 1.44 ± 0.12 h and 39.2 ± 2.09 µg h ml^{-1}. When co-administered with paracetamol, ceftizoxime attained a higher peak plasma level of 33.3 ± 1.78 µg ml^{-1} and the drug was detected in plasma above the minimum

therapeutic concentration up to 8 h post-administration. The disposition pattern followed the two-compartment open model and a significant increase was observed in the values of AUC (74.1 ± 2.01 µg h ml^{-1}) and $t_{1/2}$ (4.08 ± 0.54 h).

Patil (2010) studied effect of co-administration of ketoprofen (3 mg/kg) on pharmacokinetics of cefepime (20 mg/kg) in cow calves. Following intramuscular administration of cefepime in ketoprofen-treated cow calves, the absorption half-life ($t_{1/2ka}$), elimination half-life ($t_{1/2\beta}$), apparent volume of distribution (Vd_{area}), total body clearance (Cl_B) and mean residence time (MRT) were 0.18 ± 0.01 h, 5.36 ± 0.19 h, 0.79 ± 0.06 L kg^{-1}, 1.67 ± 0.09 ml min^{-1} kg^{-1} and 7.30 ± 0.15 h, respectively. The average values for area under plasma drug concentration-time curve [$AUC_{(0-\infty)}$] and area under first moment curve (AUMC) were 50.68 ± 5.94µg h ml^{-1} and 367.26 ± 36.00µg h^2 ml^{-1}, respectively. Significant alteration in pharmacokinetic parameters in ketoprofen-treated cow calves has not been observed compared to healthy cow calves.

Patel *et al.* (2012[b]) studied the effect of co-administration of ketoprofen (3 mg/kg) on pharmacokinetics of cefepime (20 mg/kg) in sheep. Following intramuscular administration of cefepime in ketoprofen-treated sheep, the absorption half-life ($t_{1/2ka}$), elimination half-life ($t_{1/2\beta}$), apparent volume of distribution (Vd_{area}), total body clearance (Cl_B) and mean residence time (MRT) were 0.22 ± 0.01 h, 5.22 ± 0.20 h, 0.97 ± 0.1 L kg^{-1}, 0.13 ± 0.01 L h^{-1} kg^{-1} and 6.17 ± 0.19 h, respectively. The average values for area under plasma drug concentration-time curve ($AUC_{0-\infty}$) and area under first moment curve (AUMC) were 162.37 ± 14.47 µg h ml^{-1} and 1013.18 ± 116.63 µg h^2 ml^{-1}, respectively. Significant alteration in absorption half-life and peak serum drug concentration of ketoprofen-treated sheep has been observed compared to

healthy sheep while other pharmacokinetics parameters were not significantly altered in ketoprofen-treated sheep compared to healthy sheep.

2.11 Recommended dosage regimen of cefquinome

Dinakaran *et al.* (2013) studied the pharmacokinetics of cefquinome in buffalo calves at dose rate of 2 mg/kg and reported that a single intravenous injection of 2 mg/kg may be effective to maintain the MIC upto12 h in buffalo calves against the pathogens for which cefquinome is indicated.

Dumka *et al.* (2013) studied the pharmacokinetics of cefquinome in goats following IV and IM administration at dose rate of 2 mg/kg and reported that a dosage regimen of 2 mg/kg body weight at 24 h interval following IV or IM injection will maintain the plasma levels required to be effective against the bacterial pathogens.

Tohamy (2011) studied age-related pharmacokinetics of cefquinome in sheep following a single IM administration at the doses of 1 and 10 mg/kg and reported that an optimal intramuscular dosage regimen of cefquinome would be 1 mg/kg once daily in one-month, six-months and one-year old sheep to achieve and maintain the therapeutic serum levels within safe limits.

Al-Taher (2010) studied the pharmacokinetics of cefquinome following single intramuscular administration of 1 mg/kg in camels and reported that recommended single daily dose of 1 mg/kg of cefquinome given intramuscularly can achieve quite therapeutic concentrations in serum that exceeds the minimal inhibitory concentrations against different susceptible pathogens.

2.12 Therapeutic efficacy of cefquinome

Cefquinome is a parenteral cephalosporin with broad-spectrum antimicrobial activity. Cefquinome is very potent against fastidious isolates such as *Moraxella catarrhalis* (MIC_{90}, 0.25-2 µg/ml); *Haemophilus influenzae* (MIC_{90}, 0.06-1 µg /ml),

Neisseria gonorrhoeae (MIC_{90}, 0.06-0.5 µg /ml) and *Streptococcus* species (MIC_{90}, < or = 0.03-006 µg /ml) as reported by Murphy *et al.,* 1994. Cefquinome is also active against many strains of methicillin-resistant staphylococci and enterococci (Limbert *et al.,* 1991). Cefquinome is also used for other illnesses, such as "shipping fever," a pneumonia-like illness commonly found in cattle (Shpigel *et al.*, 1997). Cefquinome has demonstrated a high resistance to enzymatic hydrolysis by -lactamases. Cefquinome is well tolerated and represents a useful alterative in clinical situations requiring a broad-spectrum antibiotic. Administration of cefquinome is effective in cows with clinical metritis (Amirids *et al*., 2003). Cefquinome was found to be effective in sows for the treatment of puerperal metritis (Heinritzi and Hagn, 1999).

Information on therapeutic use of cefquinome in domesticated animals is very scanty but in laboratory animals therapeutic efficacy of cefquinome and other fourth generation cephalosporins have been evaluated by many researchers as described in Table 2.4.

2.13 Safety and adverse effects of cefquinome

In general, compared with other class of antimicrobial agents the cephalosporins have an impressively low adverse effect profile. Hypersensitivity reactions are most common sequel and most of these reactions manifest as maculopapular skin rashes after several days of cephalosporins therapy; they may be accompanied by eosinophilia and fever. Anaphylactic reactions are uncommon with cephalosporins therapy (Kalman and Barriere, 1990). Skin rash, associated with fever and arthritis has been observed during cefaclor therapy, but this reaction is rare (Norrby, 1987).

Table 2.4. Therapeutic Efficacy of Cefquinome and other fourth generation cephalosporins.

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Coagulopathy has been reported during treatment with cefoperazone, moxalactam, cefotetan, cefmetazole and cefmandole that associated with hypoprothrombinemia, but this reaction is more of a problem in debilitated, malnourished or vitamin-K-deficient patients (Goldberg, 1987).

Renal dysfunction has been reported after cephalosporin use with transient increase in blood urea nitrogen and serum creatinine levels. Cephloridine was first implicated as a cause of nephrotoxicity (Quin, 1989). High doses (>12g per day) of cephalothin can induce renal damage in humans. Cefazolin and cefamandole can induce proximal tubular necrosis in humans and cause nephrotoxicosis in rabbits (Rankin and Sutherland, 1989).

Hepatic damage has been noted, with transient increase in serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic - pyruvictransaminase (SGPT) and alkaline phosphatase levels during treatment. Increased bilirubin and lactate dehydrogenase (LDH) levels have also been reported during the cephalosporin therapy (Kalman and Barriere, 1990). Sometimes during cephalosporins therapy, hepatomegaly has been observed, however this effect is mild and reversible upon discontinuation of the drug (Donowitz, 1989).

Gastrointestinal reactions such as nausea, vomiting and diarrhoea usually result from oral cephalosporins therapy. Tally *et al.* (1981) have reported that cefixime causes diarrhoea in 13.4 per cent and stool changes in 12.8 per cent of recipients. However these effects are usually mild and transient and only rarely necessitate discontinuation of treatment. *Clostridium difficile* toxin-associated diarrhea can occur during cephalosporin therapy and appear to be more common with cefoxitin (Kalman and Barriere, 1990). A cholecystitis like syndrome, caused by formation of biliary sludge, has been observed occasionally during ceftriaxone

therapy. It may result from precipitation of calcium salt of ceftriaxone in the gallbladder (Donowitz, 1989). Local reactions after intravenous or intramuscular administration are common with cephalosporin therapy. Pain at the site of injection, tenderness and induration are associated with intramuscular injection. Phlebitis and thrombophlebitis occur only rarely with intravenous administration. Cefquinome produce adverse effects characteristic of other extended-spectrum cephalosporins.

Jun *et al.* (2008) studied acute toxicity and cumulative toxicity of cefquinome sulphate in Mice. The mouse oral LD_{50} of cefquinome sulphate was higher than 5000 mg/kg, LD_{50} arrived at 844.03 mg/kg after intraperitoneal injection of its suspension and 95% confidence interval was 802.05 mg/kg to 887.56 mg/kg. The accumulative coefficient K was higher than 5.3 and no death occurred. The mortality of mice in test group was significantly lower than that in control group. The toxicity of domestic cefquinome sulphate is low, and the drug is safe and can be used in clinic. There is no cumulative toxicity in mice and the mice showed the obvious tolerance to this drug.

Cefquinome showed better efficacy with very few side effects. Cefquinome can be used with safety and efficacy in equids (Widmer *et al.*, 2009).

Maden *et al.* (2001) investigated biochemical and haematological side-effects of cefquinome in healthy dogs and reported that at the dose of 1 mg/kg, cefquinome did not affect haematological, blood gas, and biochemical variables in the dogs and concluded that cefquinome does not have any major side effects on biochemical and haematological variables at a dose of 1 mg/kg in dogs.

2.14 Detection methods for Cefquinome concentration in body fluids

Cephalosporins are generally assayed in biological fluids and pharmaceutical preparations by microbiological assay method or High Performance Liquid

Chromatography (HPLC). In microbiological assay method, cefquinome has been assayed in serum of camels using *Micrococcus luteus* (ATCC 9341) as test organism (Al-Taher, 2010) and in sheep (Tohamy, 2011). This technique is very slow, cumbersome, time consuming and susceptible to interface from concurrently administered antibiotics (Bowman *et al.,* 1984).

Liquid Chromatography (HPLC/UPLC/LC-MS) has become the technique of the choice for routine determination of any therapeutic agents in biological fluids. The major benefits of liquid chromatography are specificity, rapidity, sensitivity and small sample volume (Bowman *et al*., 1984 and Al-Rawithi *et al*., 2000).

Uney *et al.* (2011) developed an accurate, sensitive and least time consuming RP-HPLC method for estimation of cefquinome. The optimized method was developed using C18 (250 mm x 4.6 mm) column at room temperature with binary-gradient mobile phase with water containing 0.1% TFA as mobile phase A and ACN as mobile phase B at a flow rate of 0.9 ml/minute. UV detection was performed at 268 nm. The detection limit of cefquinome was 0.01 µg/ml.

Several studies dealing with determination of cefquinome in biological fluids (milk, plasma and bronchoalveolar lavage fluid) using HPLC have been reported (Sorensen *et al.,*2000; Maes *et al.,*2007; Zonca *et al.,*2011; Uney *et al.,*2011).

Sorensen and Snor (2000) determined the concentrations of cefquinome in raw bovine milk. The milk fat was removed by centrifugation and the cefquinome was extracted in acetonitrile. The extract was cleaned up by solid phase extraction on an octadecylsorbent. The compounds were separated by ion-paired gradient HPL Conaphenyl column with ultraviolet detection at 270 nm. The limit of detection for cefquinome was 7 µg/kg.

2.15 Pharmacology of tolfenamic acid

Tolfenamic acid (TA) is a new non-steroidal anti-inflammatory agent of the fenamate sub-group. Chemically it resembles mefenamic acid and flufenamic acid. Tolfenamic acid inhibits the biosynthesis of prostaglandins and also has an inhibitory action on prostaglandin receptors. It does not appear irritant towards the gastric mucosa, it is well tolerated by patients and it has shown good results in clinical studies, including long-term treatment of rheumatoid arthritis. Principal mechanism of action for both pharmacological and toxic effects is believed to be inhibition of cyclo-oxygenase (COX) (Linden *et al.,* 1975, 1976; McKellar *et al.,* 1994; Landoni *et al.,*1995, 1996[a, b]; Lees *et al.,* 1998). This enzyme catalyses the conversion of arachidonic acid to pro-inflammatory prostaglandins (Vane, 1971). However, TA exerts other actions at molecular and tissue levels; for example, it possesses anti-oxidant properties and inhibits some actions of prostaglandins as well as their synthesis. Moreover, *in vitro* studies in man have indicated that it produces dual inhibition of COX and 5-lipoxygenase (5-LO). Tolfenamic acid is a potent inhibitor of *in vitro* serum thromboxane $(Tx)_{B2}$ synthesis in dogs and calves (McKellar *et al.,* 1991, 1994; Landoni *et al.,* 1996[a]; Lees *et al.,* 1998; Sidhu *et al.,* 2005).

Tolfenamic acid has been used extensively in dog, cat, calf, human and pig medicine. It has used clinically as an anti-inflammatory and analgesic agent for locomoter diseases in the dogs (Robertson and Taylor, 2004) and febrile syndromes and post-operative pain in cats (Slingsby and Waterman-Pearson, 2000).

Pharmacokinetics of tolfenamic acid has been reported in dogs (McKellar *et al*., 1991), horses (Jaussaud *et al.,* 1991), calves (Landoni *et al.,* 1996[a]), camel (Wasfi *et al.,* 1998), man (Pentikainen *et al.,* 1981) and in buffalo calves (Dinakaran and Dumka, 2012).

2.15.1 Safety of tolfenamic acid

Patel *et al.* (2011[a]) studied safety of tolfenamic acid (4 mg/kg) following repeated intramuscular administration in wistar rats and reported that repeated administration of tolfenamic acid in wistar rats was safe based on evaluation result of haematological and serum biochemical (Blood glucose, AST, ALT, ALP, total bilirubin, serum total protein, serum albumin, globulin, creatinine, urea and uric acid) parameters as well as gross lesions and histopathology of liver, kidney, stomach, intestine, heart, spleen, joint cartilage and muscles of injection site. Significant changes in absolute and relative organ weights in tolfenamic acid treated rats have not been observed.

Herbal Bioenhancer

2.16 Trikatu - Herbal Bioenhancer

The concept of bioenhancers or biopotentiators is new to the modern science. It was first time reported by Bose in 1929, who described the increase in the antiasthmatic effects of vasaka (*Adhatoda vasica*) leaves by the addition of long pepper to it. A bioenhancer is an agent capable of enhancing the bioavailability and efficacy of a drug with which it is co-administered, without any pharmacological activity of its own at the therapeutic dose used. They tend to decrease the dose of active drug required for the optimal endpoint of the treatment strategy, bypassing the need to use injectable routes of drug administration to a larger extent, might help in overcoming the resistance to antimicrobials and saving the precious raw materials for the manufacturing of medicines. Such fixed drug combinations (FDCs) are economically viable too. The concept of bioenhancer is called *Yogvahi* in Ayurveda. Synergism, that is, increase in the action of one biomolecule by another unrelated chemical is the hallmark of polyherbal formulations of bioenhancers.

Taking leads from ayurveda and other traditional ways of medicine is nothing new for a modern researcher. Origin of about 75% of antimicrobial and 60% of anticancer drugs approved for clinical use from 1981 to 2002 could be traced back to nature (Patwardhan *et al*., 2010). It has taken lead from the use of "trikatu" as a bioenhancer from ayurveda and successfully applied it to various modern medicines to enhance their bioavailability.

Ancient Indian medical texts such as Charak Samhita and Sushruta Samhita (documented in about 1000 B.C) have depicted use of plants and polyherbal formulations for health care and many of them are still in use in Indian system of medicine. One of such polyherbal formulation, Trikatu, (a sanskrit word meaning three acrids) a combination of *Piper longum*, *Piper nigrum* and *Zingiber officinale* (in the ratio of 1:1:1 w/w/w). The Ayurvedic materia medica mentions trikatu as an essential ingredient either in combination or alone of many formulations used for a wide range of diseases (Atal *et al.*, 2010). The active principle of trikatu is an alkaloid piperine or 1 – peperoyl piperidine. Several studies have reported enhancement of blood levels of drugs like isoniazid, indomethacin, ampicillin, ciprofloxaxin, norfloxacin, pefloxacin and oxytetracycline when coadministered with trikatu or piperine (Karan *et al*., 1998; Karan *et al*., 1999[a]; Bhise and Pore, 2002; Singh *et al*., 2005; Janakiraman and Manavalan, 2008[ab]; Dama *et al*., 2008). Hence the study to observe effect of trikatu on pharmacokinetics of cefquinome was conceptualized.

Piperine (1-piperoyl piperidine) is the major active pungent constituent in various species of Piper species (*Piperaceae*) (Johri and Zutshi, 1992; Finar, 1975). Among these, the black pepper (*Piper nigrum Linn.*) and long pepper (*Piper longum Linn.*) are well known and widely used by the large number of people all over the world. They are also used as ingredients in the folkloric medicine for treatment of

gastrointestinal tract disorders since ancient time (Pei, 1983). The piperine contents in *P.nigrum Linn* and *P. longum Linn* are 3-9% and 3-5% (on dry weight basis) respectively (Platel and Srinivasan, 2001). It can be isolated from the oleoresin of the pepper by extraction from the powdered fruit of the plant by dichloromethane or alcohol and purified in crystalline powder (Bhat and Chandrasekhara, 1985).

2.16.1 Physico-chemical properties of piperine

Chemical name: The chemical name of piperine is (E, E)-1-[5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl] piperidine.

Molecular formula: $C_{17}H_{19}NO_3$

Molecular weight: 285.34

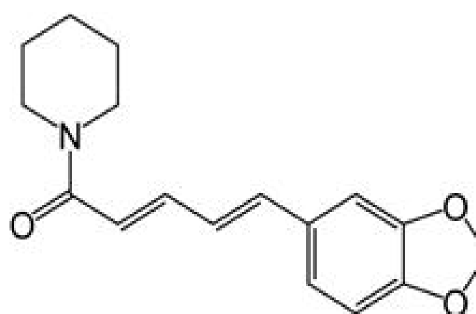

Figure 2.4 The chemical structure of Piperine.

Piperine Structure

Piperine structure (Figure-2.4) consists of three important components including methylene dioxy phenyl (MDP) ring, side chain with conjugated double bonds and a basic piperidine moiety attached through a carbonyl amide linkage to side chain (Finar, 1975; Merck, 2000).

2.16.2 Pharmacokinetics of piperine

It has been reported that after giving piperine to the male albino rats, regardless of the dose and route of administration, about 97 % of piperine was absorbed in the gastrointestinal tracts. Only 3% was excreted as piperine in faeces but no free form of piperine was excreted in the urine. The piperine transformation in duodenal segment is higher than jejunum and ileal segments (Bhat and Chandrasekhara, 1986).

Piperine is biotransformed in the liver by the hepatic cytochrome P-450 dependent monooxygenase. It is biotransformed to piperic acid, piperonylic acid, piperonyl alcohol, piperonal and vanillic acid and their conjugated derivatives. The probable reactions of piperine metabolism are demethylation, glucuronidation and sulphation of piperine. After administration of the piperine to rats with regardless to the route of administration, trace to small amount of piperine could be detected in serum, spleen, kidney and liver from 30 minutes to 24 hour but beyond 24 h piperine was not detected in any above tissue. After oral administration of piperine (170 mg/kg) to rats, the metabolites in urine (0-96 h) were identified to be piperonylic acid, piperonyl alcohol, piperonal, and vanillic acid in the free form, whereas only piperic acid was detected in bile between 0-6 h (Bhat and Chandrasekhara, 1987).

The kidney appears to be the major excretion route for piperine metabolites in rats. Based on these results, a pathway for the biotransformation of piperine in rats has been proposed (Figure: 2.5). It was speculated that piperine was absorbed into portal vein, which was first hydrolyzed to piperic acid and piperidine mainly in the liver, and then the former was transformed to piperonylic acid after successive oxidation of the side chain (Bhat and Chandrasekhara, 1987).

Bajad *et al.*, (2003) reported differences in piperine metabolism in rats and humans, a new major urinary metabolite was detected in rat urine and plasma using HPLC and characterized as 5-(3,4-methylene dioxy phenyl)-2,4-penta dienoic acid-N-(3-yl propionic acid)-amide. Data suggested that piperine is absorbed very fast across the intestinal barrier. It may act as a polar molecule and form a polar complex with drugs and solutes.

2.16.3 Effect on drug metabolizing enzymes

The effect of piperine on the drug metabolizing enzymes has been studied extensively both *in vitro* and *in vivo* (Atal *et al.*, 1985 and Reen and Singh, 1991). Piperine showed to inhibit the activity of the enzymes aryl hydrocarbon hydroxylase (AHH), ethyl morphine-N-de methylase, 7-ethoxy coumarin-O-de ethylase (7ECDE) and 3-hydroxy-benzo (a) pyreneglucuronidase in rat post mitochondrial supernatant in vitro in a dose dependent manner. These studies indicated that piperine was a non-specific inhibitor of drug metabolizing enzymes (Atal *et al.*, 1985).

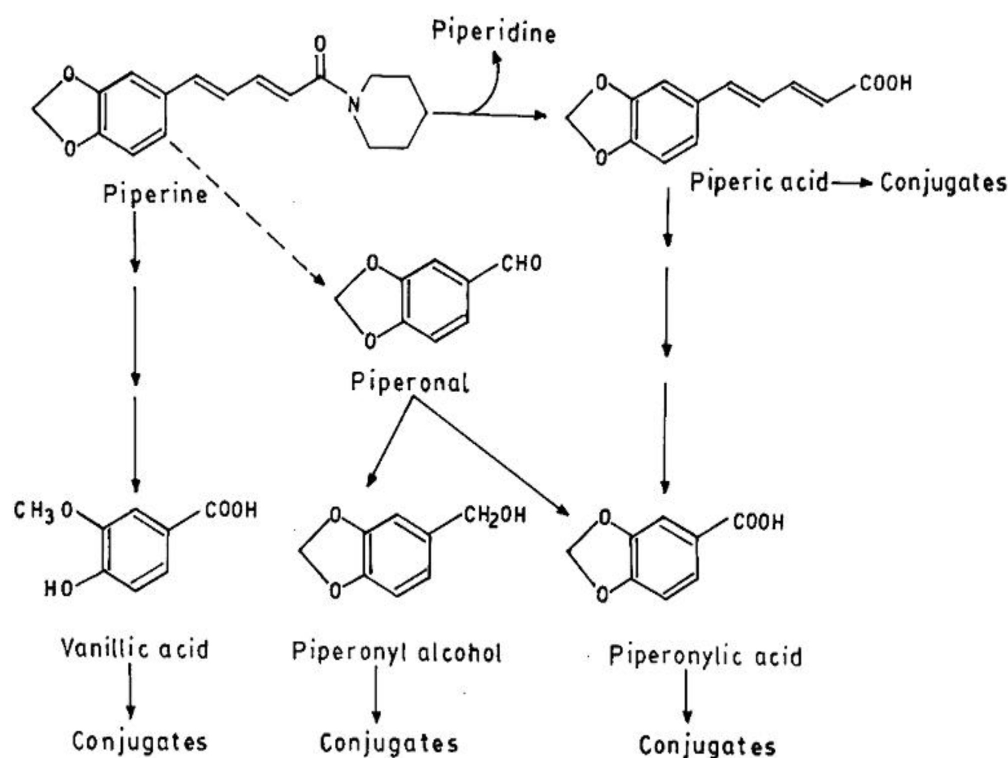

Figure 2.5: Biotransformation of Piperine

A single oral administration of piperine (125 mg/kg body weight) in rats strongly inhibited the hepatic aryl hydrocarbon hydroxylase (AHH) and UDP-glucuronyl transferase activities. The maximal inhibition of hepatic aryl hydrocarbon hydroxylase (AHH) of rats observed within 1 hour and by 6 hours after

administration, the enzymatic activity returned to the normal control value (Atal *et al.*, 1985).

In vitro and *in vivo* modulation of drug metabolizing enzymes by piperine was investigated in microsomes of rat and guinea pigs (Khajuria *et al*., 1998). These studies indicated that piperine caused concentration related inhibition of NADPH-dependent cytochrome P-450 oxidase enzymes which play a central role in disposition, steady state balance of drugs and xenobiotics. In addition, piperine is a strong inhibitor of UDP-glucoronyltransferase enzyme and impaired down regulation of Cytochrome P-450 1A1 gene expression in the rat hepatoma 5 L cell lines. It was due to direct interaction of the alkaloid with Cytochrome P-4501A1 enzyme at post – translation level (Guido *et al*., 1998).

Furthermore, piperine showed the various effects in liver and small intestine glucuronidation in rats. Feeding of piperine to the rat at the dose of 100 mg/kg body weight caused an increase in hepatic microsomal cytochrome P-450 and cytochrome b, NADPH-cytochrome Creductase, 5 benzphetamine N-demethylase, aminopyrine N- demethylase and aniline hydroxylase within 24 hours after treatment. Rats were given piperine intraperitoneally (10 mg/kg body weight) which had no effect on the activities of the aforementioned enzymes, while at the doses of 800 mg/kg and 100 mg/kg orally and intraperitoneally respectively, had significantly decreased in the activities of the mentioned enzymes except cytochrome B and NADP- 5 cytochrome Creductase (Dalvi and Dalvi, 1991).

Treatment of rat with piperine (1.4 mmol/kg, 3 days intraperitoneal injections) resulted in an approximate two-fold increase in total liver microsomal P450 content relative to that in uninduced animals. These results demonstrate that piperine treatment suppressed P4502E1 expression and enhanced 2B and 1A expression,

whereas this agent failed to affect hepatic epoxide hydrolase and glutathione S-transferase (GST) expression (Kang *et al.*, 1994).

2.16.4 Piperine as bioavailability enhancer

Piperine, obtained from the oleoresin in the peppercorns is by far the most studied and researched Bioenhancer. Modern researchers are increasingly showing interest toward the improvement of bioavailability of a large number of drugs by addition of various herbs with bioenhancing properties. Exhaustive ayurvedic literature search led to the identification and isolation of piperine, an alkaloid, from *Piper longum*—the world's first purified bioenhancer molecule (Atal *et al.*, 1985). Piperine obtained from botanical sources is about 98% pure.

Potential of piperine to increase the bioavailability of drugs is of great clinical significance. A concise mechanism responsible for its bioavailability enhancing action is poorly understood. Atal *et al*. (1981) have evaluated the scientific basis of enhancement of drug bioavailability by piperine and concluded that piperine is a potent inhibitor of drug metabolism. Hence increases the bioavailability of drugs either by promoting rapid absorption from the gastrointestinal tract, or by protecting the drug from being metabolized in its first passage through the liver after being absorbed, or by a combination of these two mechanisms. Influence of piperine on bioavailability of various antibiotics in different animals is summarized in Table 2.5. Several studies have reported enhancement of blood levels of drugs like vasicine, sparteine, phenytoin, propranolol, theophylline, rifampicin, sulphadiazine and tetracycline when co-administered with trikatu or piperine (Atal *et al*., 1981; Zutshi *et al*., 1985; Bano *et al*., 1987; Bano *et al*., 1991; Mathur *et al*., 1998)

The effect of trikatu (piperine) on the bioavailability and pharmacokinetics of isoniazid in rabbits was evaluated by Karan *et al*. (1998). In a crossover study, ten

rabbits were administered either single dose (orally) of isoniazid (14 mg/kg) alone or in combination with trikatu [piperine (10 mg/kg)]. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 4, 6, 9 and 12 hours after drug administration and assayed for isoniazid by fluorimetric technique. A washout period of 7 days was allowed between the two treatments. Co-administration of trikatu (piperine) significantly reduced the C_{max} (5.48 ± 0.75 µg/mL Vs 8.42 ± 0.85 µg/mL) and AUC (15.04 ± 3.64 µg. h/mL.Vs 24.76 ± 4.03 µg·h/mL) of isoniazid.

Karan *et al*. (1999[a]) investigated the influence of co-administration of piperine (500 mg/kg P.O.) on pharmacokinetic of indomethacin (7 mg/kg) in rabbits. It is evident that in the absorption phase, trikatu significantly enhanced plasma concentrations of indomethacin at 0.5, 1, 1.5, 2 and 4 hours after drug administration, while no significant difference was observed in the elimination phase. There was no significant difference in any of the pharmacokinetic parameters measured except C_{max}, which was significantly higher in the trikatu treated group.

The effect of single and multiple doses of a herbal preparation trikatu, an ayurvedic prescription, on the bioavailability and pharmacokinetics of rifampicin was studied in rabbits (Karan *et al*., 1999[b]). Rabbits (n=10) were administered a single dose of rifampicin (24 mg/kg, P.O.) alone or in combination with a single dose of trikatu (500 mg/kg, P.O.). In both studies, blood samples were collected at 0, 0.5, 1, 1.5, 2, 4, 6, 9 and 12 h after drug administration and assayed for rifampicin. In animals treated with single dose of trikatu there was a significant decrease in the peak plasma concentration (C_{max}) of rifampicin ($P<0.05$). Multiple doses of trikatu also reduced the C_{max} and delayed the T_{max} of rifampicin although not to a statistically significant level. Other pharmacokinetic parameters of rifampicin were not significantly altered. The results suggest that co-administration of trikatu does not

influence the extent of bioavailability (AUC) but reduces the rate of bioavailability (C_{max}) of rifampicin and this latter effect may reduce the efficacy of rifampicin therapy.

Hiwale *et al.,* (2002) evaluated effect of co-administration of piperine on pharmacokinetics of - lactam antibiotics (amoxicillin trihydrate, cefotaxime sodium and cefadroxil monohydrate) in rats. Amoxycillin trihydrate, cefotaxime sodium, cefadroxil monohydrate and Piperine were prepared as suspension in carboxy methyl cellulose (CMC) (0.5% w/v) using pestle and mortar for oral administration to rats. The improved bioavailability is reflected in various pharmacokinetic parameters viz. T_{max}, C_{max}, $t_{1/2}$ and AUC, of these antibiotics are described in table 2.5. The increased bioavailability could be attributed to the effect of piperine on microsomal metabolizing enzymes or enzymes system.

Bhise and Pore, (2002) investigated the influence of co-administration of piperine (10 mg/kg) on pharmacokinetics of ciprofloxacin (80 mg/kg) in rabbits. The pharmacokinetic values for ciprofloxacin alone and along with piperine after oral administration were: $t_{1/2}$ (3.61 ± 0.05 and 4.008 ± 0.042 h), AUC (94.088 ± 1.7 and 160.24 ± 1.07 µg.h/mL), C_{max} (13.42 ± 0.34 and 22.60 ± 0.258 µg/L) and T_{max} (2.06 ± 0.16 and 1.55 ± 0.025 h) respectively.

Walunj, (2008) investigated the influence of co-administration of trikatu on pharmacokinetics of gatifloxacin (10 mg/kg, PO) in sheep. The pharmacokinetic values for gatifloxacin alone and along with trikatu after oral administration were: $t_{1/2}$ (4.05 ± 0.19 and 4.39 ± 0.16 h), AUC (12.44 ± 0.26 and 15.34 ± 0.31 µg.h/mL), C_{max} (1.62 ± 0.02 and 1.80 ± 0.03 µg/L) and T_{max} (2.00 ± 0.0 and 12.00 ± 0.0 h) respectively.

Singh *et al.,* (2005) reported alteration in pharmacokinetic profile of orally administered oxytetracycline (10 mg/kg body weight) following post oral treatment of *Piper longum* (15 mg piperine equivalent/kg body weight for 7 days) in White Leghorn hens. The pharmacokinetic data revealed that *Piper longum* treated animals had significantly higher area under curve (AUC), area under the first moment of plasma drug concentration-time curve (AUMC) and mean residential time (MRT). Prior administration of *Piper longum* significantly reduced elimination rate constant ( ) and increased elimination half-life (t½). The total body clearance (Cl_B) reduced by 21% whereas total duration of pharmacological effect (t_d) increased by 29%. The treatment with *Piper longum* reduced loading and maintenance dose by 33.3 and 39%, respectively.

Dama *et al*., (2008) evaluated bio-enhancing effect of the, trikatu, in mountain Gaddi goats (n = 6) following oral administered pefloxacin. The findings of the study revealed a decreased plasma concentration (p>0.05) of pefloxacin following trikatu administration during the absorption phase (10, 15, 20 min post pefloxacin administration). In contrast, the plasma concentrations of pefloxacin were significantly higher at 4, 6, 8 and 12 h (during the elimination phase) of the pefloxacin administration. The findings of the investigation revealed higher values for the area under the curve, the area under the first moment of the plasma drug concentration time curve, the mean residential time, the total duration of pharmacological action and bioavailability. Trikatu treatment, however, significantly reduced the elimination half-life ($t_{1/2}$) and zero time intercept of the elimination phase. The apparent volume of distribution ($Vd_{(area)}$) based on the total area under the plasma drug concentration curve (AUC) was significantly higher in trikatu treated animals indicating a better penetration of the drug. Based on the MIC of 0.8 µg/mL of pefloxacin, a priming dose

of 6.0 mg/kg and a maintenance dose of 2.21 mg/kg are required to be administered at 8 h intervals. For practical purposes in goats this would mean a priming dose of 6 mg/kg and a maintenance dose of 2 mg/kg given by the oral route, to be repeated at 8 h intervals.

Janakiraman and Manavalan, (2008[a]) studied the effect of piperine co-administration (20 mg/kg, single dose) on oral bioavailability of ampicillin (150 mg/kg body weight, single dose) and norfloxacin (150 mg/kg body weight, single dose). There was increase in AUC and also in other pharmacokinetic parameters of rabbits administered piperine along with norfloxacin and ampicillin. Pharmacokinetic values for ampicillin (150 mg/kg) alone and along with piperine (20 mg/kg) following oral administration were: $t_{1/2}$ (1.3 ± 0.46 h), AUC (103.7 ± 0.52 µg.h/mL), C_{max} (44.6 ± 0.27 µg/mL), T_{max} (1 ± 0.31 h) and $t_{1/2}$ (1.9 ± 0.57 h), AUC (350.486 ± 0.47µg.h/mL), C_{max}(251.2 ± 0.28 µg/mL), T_{max}(1.1 ± 0.3 h) respectively. Pharmacokinetic values for norfloxacin (150 mg/kg) alone and along with piperine (20 mg/kg) following oral administration were: $t_{1/2}$ (1.75 ± 0.38 h), AUC (63.976 ± 0.51µg.h/mL), C_{max} (11 ± 0.26 µg/mL), T_{max} (3.1 ± 0.32 h) and $t_{1/2}$ (2.97 ± 0.38 h), AUC (111.695 ± 0.54 µg.h/mL), C_{max}(16.1 ± 0.27 µg/L) and T_{max} (1.1 ± 0.3 h) respectively.

The study was aimed to find out the efficacy of trikatu or its components in enhancing bioavailability of the ampicillin. The extracts of components of trikatu were prepared by soxhlation using alcohol (90%). The study was carried out in rabbits by oral administration of the extracts of the components and their combination in equal proportions (trikatu) along with above antibiotic. Blood samples were drawn at time intervals equal to three half-life of the antibiotics and their plasma concentrations were estimated microbiologically. Trikatu and its components

enhanced the bioavailability of ampicillin, trikatu has shown maximal enhancement for ampicillin (Janakiraman and Manavalan, 2008[b]).

Patel *et al*. (2011[b]) investigated the influence of co-administration of piperine on pharmacokinetic profile of gatifloxacin in layer birds. The pharmacokinetic profile of gatifloxacin (10 mg/kg body weight) along with piperine co-administration (15 mg/kg) via single oral dose in layer birds observed half-life ($t_{1/2}$), peak plasma drug concentration (C_{max}) and AUC of 4.03 ± 0.097 h, 2.14 ± 0.019 µg/mL and 17.54 ± 0.204 µg.h/mL, significantly higher than gatifloxacin alone (3.74 ± 0.073 h, 1.74 ± 0.023 µg/mL and 15.25 ± 0.219 µg.h/mL respectively). This study revealed that piperine has significant effect on the pharmacokinetics of the Gatifloxacin. There was enhancement in bioavailability (F) from 74.52 ± 1.021% (gatifloxacin alone treated group) to 85.74 ± 0.956 % (piperine co-administration with gatifloxacin treated group).

Patel (2012) investigated the influence of co-administration of trikatu on pharmacokinetic profile of levofloxacin in goats. The pharmacokinetic profile of levofloxacin (4 mg/kg body weight) along with trikatu co-administration (piperine equivalent to 20 mg/kg) in goats observed half-life ($t_{1/2}$), peak plasma drug concentration (C_{max}) and AUC of 2.07 ± 0.15 h, 0.74 ± 0.03 µg/mL and 4.15 ± 0.10 µg.h/mL, significantly higher than levofloxacin alone (0.95 ± 0.065 h, 0.60 ± 0.031 µg/mL and 2.89 ± 0.139 µg.h/mL respectively). This study revealed that trikatu has significant effect on the pharmacokinetics of the levofloxacin. There was enhancement in bioavailability (F) from 21.49 ± 1.60 % (levofloxacin alone treated group) to 30.16 ± 1.31 % (trikatu co-administration with levofloxacin treated group).

Nduka *et al*. (2013) investigated the effects of *Zingiber officinale* on the plasma pharmacokinetics and lung penetrations of ciprofloxacin and isoniazid and reported that ginger significantly increased the area under the concentration-time curve of ciprofloxacin and significantly decreased the area under the concentration-time curve of isoniazid. This study revealed that ginger enhanced the penetration of ciprofloxacin and isoniazid into the lung tissues.

Table 2.5: Pharmacokinetic parameters of antibiotics with Trikatu in animals.

The present study was undertaken to determine the effect of co-administration of tolfenamic acid and bioenhancer trikatu on pharmacokinetics of cefquinome and safety of cefquinome alone and in combination with tolfenamic acid in sheep.

3.1 Experimental animals

The study was conducted on six Patanwadi sheep of 2-3 years weighing between 30 and 35 kilograms. The animals were obtained from and maintained at the Instructional Farm, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand. They were kept under constant observation for two weeks prior to commencement of the experiment. During this period they were subjected to clinical examination in order to exclude the possibility of any disease. The animals were then housed in separate pen and were provided standard ration. Water was provided *ad libitum*. All necessary managemental procedures were adopted to keep the animals free from stress. The experimental protocol for general procedure and use of animals for conducting the present study has been reviewed by the Institutional Animal Ethics Committee (IAEC) and submitted to CPCSEA.

3.2 Drugs and chemicals

Cefquinome suspension (25 mg/ 50mL; Cobactan® 2.5%, Intervet India Pvt. Ltd., Pune, India.) and tolfenamic acid injection (40 mg/ml; Maxxtol®, Intas Pharmaceutical Ltd. Ahmedabad, India.) were procured from pharmacy. Cefquinome technical grade powder was procured from was purchased from Sigma-Aldrich. acetonitrile, methanol, trifluroacetic acid and perchloricacid (about 70%) (Analytical grade) and water of HPLC grade were purchased from Merck Limited, Mumbai. Trikatu was purchased from Dhanvantri Pharma Ltd., Anand, Gujarat, India.

3.3 Plan of work

The present study was conducted broadly in three different phases (Table 3.1).

Phase I

To study pharmacokinetics of cefquinome administered (2 mg/kg) alone through IM route or co-administered with IM injection of tolfenamic acid (2 mg/kg) in sheep.

Phase II

To evaluate bioenhancing effect of trikatu (2 g/kg, PO) on pharmacokinetics cefquinome (2 mg/kg, IM) in (7 days pretreated) sheep.

Phase III

To evaluate the safety of cefquinome alone (2 mg/kg, IM, repeated at 24 h for 5 days) and in combination with tolfenamic acid (2 mg/kg, IM, repeated at 24 h for 5 days) in sheep.

3.4 Studies on pharmacokinetics of cefquinome alone and in combination with tolfenamic acid in sheep (Phase I)

Present study was conducted in a cross over design with an interval of fifteen days between successive administrations of the drugs. Six healthy sheep (S1, S2, S3, S4, S5 and S6) were employed to investigate the pharmacokinetics of cefquinome alone (intramuscularly) and in combination with tolfenamic acid (intramuscularly) administration in sheep as per protocol depicted in Table 3.1.

Table 3.1: Experimental protocol to study the effect of tolfenamic acid and bioenhancer trikatu on pharmacokinetics of cefquinome (IM) and safety of cefquinome (IM) in combination with tolfenamic acid in sheep.

Phase	Treatments	Animal No.
I	Cefquinome (IM)	S1
	Cefquinome (IM)	S2
	Cefquinome (IM)	S3
	Cefquinome (IM) + Tolfenamic acid (IM)	S4
	Cefquinome (IM) + Tolfenamic acid (IM)	S5
	Cefquinome (IM) + Tolfenamic acid (IM)	S6
II	Pulverized trikatu (2 g/kg, PO) + Cefquinome (IM)	S1, S2, S3, S4, S5 and S6
III	Cefquinome (IM) + Tolfenamic acid (IM) for 5 Days	S1, S2, S3, S4, S5 and S6
	All six animals received Cefquinome (2 mg kg^{-1}, repeated at 12 h) and Tolfenamic acid (2 mg kg^{-1}, repeated at 24 h) for 5 days.	

*Each animal received above three types of treatments with an interval of fifteen days.

3.4.1 Administration of cefquinome and tolfenamic acid

Cefquinome was administered at a dose rate of 2 mg/kg of body weight. Injectable cefquinome (25 mg/50 mL) was used for intramuscular administration.

Tolfenamic acid was administered at the dose rate of 2 mg/kg of body weight intramuscularly in deep gluteal muscle.

3.4.2 Collection of blood samples

Blood samples (2 mL) were collected from intravenous catheter (Venflon, 22 × 0.9 × 25 mm) fixed into the contralateral jugular vein. Following intramuscular administration blood samples were collected at 0 minute (before drug administration), and at 0.083 (5 minutes), 0.166 (10 minutes), 0.25 (15 minutes), 0.5 (30 minutes), 0.75 (45 minutes) and at 1, 2, 4, 8, 12, 18, 24 and 36 hours.

The blood samples were transferred to clean sterilized heparinized test tubes. Plasma was separated soon after collection by centrifugation at 3000 revolution per min (rpm) for 10 minutes at 10°C (Eppendorf 5804 R, Germany). Separated plasma samples were transferred to labeled cryovials and stored at -35 °C until assayed for cefquinome concentration using High Performance Liquid Chromatography (HPLC) procedure which was done within 24 to 36 h.

3.5 Evaluation of bioenhancing effect of trikatu on pharmacokinetics of cefquinome following oral administration in sheep.

The study was conducted in a cross over design with an interval of fifteen days between successive administrations of the drugs. Six healthy sheep (S1, S2, S3, S4, S5 and S6) were employed to investigate bioenhancing effect of trikatu on pharmacokinetics of cefquinome following single dose oral administration as per protocol shown in Table 3.1 Phase II.

3.5.1 Administration of cefquinome and trikatu in sheep.

Cefquinome was administered at a dose rate of 2 mg/kg of body weight intramuscularly. Pulverized powder of trikatu suspended in water was given orally

through esophageal tube for 7 days (pretreatment) before administration of cefquinome. Minimum of 15 days of washout period was maintained between treatments.

3.5.2 Collection of blood samples

The schedule of blood samples (2 mL) collection and preservation following cefquinome administration via intramuscular route was similar to Phase I

3.6 Cefquinome Assay

Cefquinome was assayed in plasma by adopting procedure as described by Uney *et al*., (2011) with minor modifications as described below.

3.6.1 Apparatus

The high performance liquid chromatography apparatus of Lab alliance (USA) comprising quaternary gradient delivery pump (model AIS 2000) connected with Autosampler (model Sykam S 5200) and UV detector (model 500) were used for assay. Chromatographic separation was performed by using reverse phase C_{18} column (Whatman, PARTISIL 5 ODS-3 RAC-II; 4.6 × 100 mm ID) at room temperature. The HPLC data integration was performed using software Clarity (Version 2.4.0.190).

3.6.2 Chromatographic conditions

The mobile phase consisted of water containing 0.1% TFA as mobile phase A and ACN as mobile phase B in the gradient flow as shown in Table 3.2. Mobile phase was filtered by 0.45 µm size filter (Ultipor N66 Nylone 6,6 membrane, PALL Pharmalab filtration Pvt., Ltd., Mumbai) and degassed by ultra-sonication. The mobile phase was pumped into column at a flow rate of 1.5 mL/min at an ambient temperature. The effluent was monitored at 270 nm wavelength.

Table 3.2: HPLC mobile-phase gradient conditions for analysis of cefquinome

Time (min)	**Flow rate** (ml/min)	**% A** (0.1% Trifluoroacetic acid in water)	**% B** (Acetonitrile)
Initial	1.5	90	10
1	1.5	10	90
4	1.5	10	90
5	1.5	90	10

3.6.3 Extraction of Cefquinome from Plasma

Two hundred microlitre (0.2 mL) of plasma sample was taken in micro-centrifuge tube (2.0 mL capacity). Methanol (400 µl) was added in order to precipitate plasma proteins. The mixture was vortexed for 1 minute and centrifuged at 10000 rpm for 5 minutes at 10°C (Eppendorf 5804 R, Germany). The supernatant was decanted in clean sterile micro centrifuge tube and 20 µL supernatant was injected into the loop injector using Autosampler (Sykam S5200).

3.6.4 Standardization and partial validation of assay

3.6.4.1 Preparation of standard curve

Initially, stock solution was prepared by dissolving 10.0 mg of cefquinome pure API grade powder in 100 ml HPLC water to get cefquinome concentration of 100 µg/ml. Known concentrations of cefquinome in plasma were prepared by diluting the stock standard with diluent or drug-free sheep plasma which was used to prepare standard cefquinome concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 µg/mL in diluent and plasma (Table 3.3). Twenty microliter of each standard prepared in

diluent was injected into the loop injector using Autosampler (model Sykam S 5200) whereas, each standard prepared in plasma was then treated by procedure as described earlier for extraction of cefquinome from plasma.

Individual standard curve was prepared using the final dilution in plasma by plotting the area of peak of cefquinome standard and the drug concentration at abscissa (Figure 3.1). The assay was sensitive, reproducible and its linearity was observed from 0.1 to 10 µg/mL with mean correlation coefficient (r^2) > 0.999. The limits of detection and limits of quantification were determined to be 0.05 and 0.1 µg/mL, respectively. Quantification of cefquinome in plasma sample was done by reference to the resultant standard curve.

Table 3.3: Procedure for preparation of cefquinome standards in diluent and plasma of sheep.

10 mg cefquinome + 100 mL HPLC Water ⟶ Stock solution (100 µg/mL)

Diluent or Drug free plasma	+	Solution	⟶	Final solution (concentration)
900 µL	+	100 µL Stock solution	⟶	Standard A (10 µg/mL)
500 µL	+	500 µL Standard A	⟶	Standard B (5 µg/mL)
500 µL	+	500 µL Standard B	⟶	Standard C (2.5 µg/mL)
900 µL	+	100 µL Standard A	⟶	Standard D (1 µg/mL)
500 µL	+	500 µL Standard D	⟶	Standard E (0.5 µg/mL)
500 µL	+	500 µL Standard E	⟶	Standard F (0.25 µg/mL)
900 µL	+	100 µL Standard D	⟶	Standard G (0.1 µg/mL)

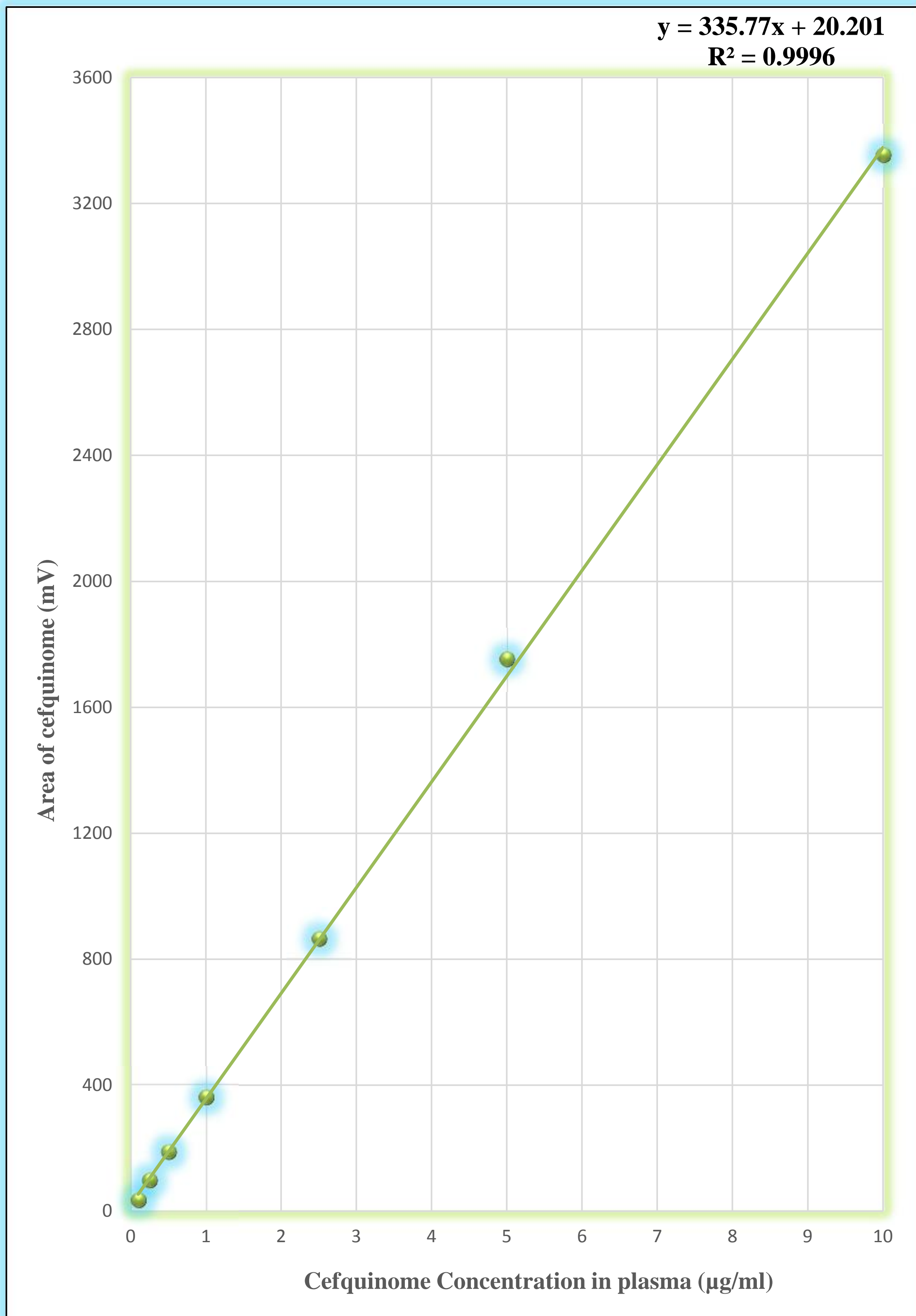

Figure 3.1: Standard curve of cefquinome in drug free plasma of sheep

3.6.4.2 Absolute recovery of cefquinome from plasma

Cefquinome standards of 0.25, 1, 2.5 and 5 µg/ml were prepared in diluents and 20 µL of each standard was injected into the loop injector using Auto sampler (model Sykam S 5200) to know the response in terms of area of peak. Similar standards of the drug in plasma were prepared and treated by the procedure as described earlier. The supernatant was decanted in clean sterile micro centrifuge tube and 20 µL supernatant was injected into loop injector using Autosampler (model Sykam S 5200) to know the response in terms of area of peak. The absolute recovery of cefquinome was measured by comparison of the areas of peak of cefquinome standards in diluents and plasma containing equivalent concentrations of the drug. The values of percent recovery of cefquinome from plasma with the final concentration of 0.25, 1, 2.5 and 5 µg/ml were mentioned in Table 3.4.

3.6.4.3 Accuracy and precision

Plasma samples (*n*=5) with the final concentration of 0.25, 1, 2.5 and 5 µg/ml for cefquinome were extracted according to the procedure mentioned above. To fulfill the requirement of partial validation of modified method, intraday and interday precision and accuracy were evaluated.

Precision and accuracy of the assay were assessed in conjunction with the linearity for each standard concentration (Table 3.4). Precision of an analytical method describes the closeness of individual measures of cefquinome when the procedure is applied repeatedly. Accuracy describes the closeness of mean test results obtained by the method to the true concentration. The intra-day assay precision and accuracy were estimated by analyzing six replicates at four different QC levels, i.e. 0.25, 1, 2.5 and 5 µg/ml. The inter-day assay precision was determined by analyzing

the four levels QC samples on three different runs. The criteria for acceptability of the data included accuracy within ±15% standard deviation (SD) from the nominal values and a precision of within ±15% relative standard deviation (RSD) (US DHHS *et al*., 2001). Accuracy and precision data for intra and inter-day plasma samples are presented in Table 3.4. The assay values on both occasions (intra and interday) were found to be within the accepted variable limits. Precision and accuracy of the assay in conjunction with the linearity indicates good accuracy of the assay with coefficient of variance (C.V.) less than 9.13 %.

Table 3.4: Intraday and interday precision, accuracy and recovery from the determination of cefquinome in plasma of sheep (n = 6)

Added concentration (µg/mL)	Measured concentration (µg/mL) Mean ± S.D.	CV %	Accuracy (%)	Recovery (%)
Intra-day				
5	4.88 ± 0.03	0.59	97.67	97.27
2.5	2.41 ±0.03	1.44	96.53	96.20
1	0.92 ± 0.08	9.13	92.02	93.81
0.25	0.22 ± 0.01	4.84	86.51	85.52
Inter-day				
5	4.85 ± 0.11	2.20	96.95	96.55
2.5	2.41 ± 0.02	0.69	96.25	95.92
1	0.92 ± 0.04	4.30	91.62	93.40
0.25	0.21 ± 0.01	5.63	85.15	82.74

3.7 Pharmacokinetic analysis

The various pharmacokinetic parameters were calculated from plasma concentration of cefquinome using software PK solution (version 2.0).

PK Solutions relies on the use of non-compartmental method of analysis for the estimation of pharmacokinetic parameters. Two non-compartmental techniques were employed. One technique is based on the estimation of the area associated with the curve described by the concentration-time profile. In this case, the classical trapezoidal rule is used to compute the area under the curve (AUC). The second non-compartmental technique was based on the method of residuals (also called curve stripping or feathering) which resolves a curve into a series of up to three exponential terms corresponding to the absorption, distribution and elimination phases occurring during the time course of the drug in the blood. These exponential terms were used to calculate the various single and multiple dose pharmacokinetic parameters following well established textbook calculations. The curve stripping approach assumes that the disposition phases of the drug follow apparent first-order rate processes, which is evidenced by linearity in the terminal portion of a semi-log plot. This is, in fact, the case for the overwhelming majority of drugs, making PK solutions a widely useful tool.

3.8 Formula used by 'PK Solution 2.0' software

3.8.1 General disposition parameters and constant

A) Dose Amount: D

B) Exponential Summation: Expression for sum of 1st order kinetic terms.

$$C = \sum C_n e^{-\lambda_n t}$$

C) Y-Intercept: Coefficient of each exponential term: C_n

D) Slope

$$s = \frac{-\lambda_n}{2.303}$$

E) Rate constant

$$\lambda_n = -2.303s$$

F) Elimination rate constant $\}_z$

G) Half-life

$$t_{½} = \frac{0.693}{\lambda_n}$$

3.8.2 Descriptive curve parameters

A) C $_{\text{initial}}$

Initial concentration extrapolated to time zero for i.v. dose

$$C_0 = \sum C_n$$

B) C_{max} (obs) = maximum concentration observed

C) T_{max} (obs) = Time at maximum concentration observed

3.8.3 Curve area calculation

A) $AUC_{(0-t)\ (obs\ area)}$

Trapezoid calculation of AUC using observed data points only (not extrapolated to infinity).

$$AUC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i + C_{i+1})$$

Where, n is the number of data points.

B) $AUC_{(0-\infty)}$

Total AUC computed by combining

$$AUC_{\infty} = AUC_{(0-t)} + \frac{C_n}{\lambda_z}$$

Where, C_n is the last concentration.

3.8.4 Statistical moment calculations

A) AUMC

Calculation of total area under the first-moment curve by combining trapezoid calculation of AUMC $_{(0-t)}$ and extrapolated area

$$AUMC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i t_i + C_{i+1} t_{i+1}) + \frac{C_{last} \cdot t_{last}}{\lambda_z} + \frac{C_{last}}{\lambda_z^2}$$

B) MRT

Mean residence time calculated using trapezoid area calculations extrapolated to infinity.

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

Where, both area terms use trapezoidal calculations.

3.8.5 Volume of distribution calculations

A) $V_{d(area)}$: Apparent volume of distribution based on trapezoid AUC (area) and elimination rate. For intravenous, complete absorption (F=1) is assumed.

$$V = \frac{FD}{AUC_{\infty} \lambda_z}$$

B) V_{ss}

Apparent volume of distribution at steady state estimated graphically from trapezoidal total area measurements.

$$V_{ss} = \frac{D \cdot [AUMC_{\infty}]}{[AUC_{\infty}]^2}$$

3.8.6 Systemic clearance calculations

A) CL

Systemic clearance based on trapezoid AUC (area). For intravenous, complete absorption (*F*=1) is assumed.

$$CL = \frac{FD}{AUC_{\infty}}$$

3.8.7 Two-compartment open model micro-constants

A) K_{12}: Micro-constant calculated using exponentials. (Applies to 2 compartment i.v. dose data only)

$$k_{12} = \lambda_1 + \lambda_z - k_{21} - k_{10}$$

B) K_{21}: Micro-constant calculated using exponentials. (Applies to 2 compartment i.v. dose data only)

$$k_{21} = \frac{C_1\lambda_z + C_z\lambda_1}{C_1 + C_z}$$

3.9 Studies on safety assessment of the cefquinome in combination with tolfenamic acid in sheep

The study was conducted in a cross over design with an interval of fifteen days between successive administrations of the drug/s. Six healthy sheep (S1, S2, S3, S4, S5 and S6) were employed to assess safety of multiple intramuscular administration of cefquinome (2 mg/kg) in combination with tolfenamic acid (2 mg/kg) repeated at 24 h interval for 5 days in sheep as per protocol depicted in Table 3.1.

3.9.1 Administration of cefquinome and tolfenamic acid

Animals (S1, S2, S3, S4, S5 and S6) were administered with cefquinome (2 mg/kg) in combination with tolfenamic acid (2 mg/kg) following intramuscular route repeated at 24 h for 5 days. All animals were observed for any side effects or clinical abnormalities during the period of experiment.

3.9.2 Collection of blood samples

Blood samples were collected before administration of the drug/s which served as control (day 0). After administration of drug/s, blood samples were collected at day 1st, 2nd, 3rd, 4th and 5th from jugular vein (before administration of drugs) into sterile tubes for hematological and serum biochemical analysis. Blood samples (2 mL) collected in heparinized test tubes were utilized for hematological evaluation, whereas blood samples (2 mL) collected in centrifuge tubes without anticoagulant were allowed to clot at room temperature. Serum was harvested by centrifugation at 3000 rpm for 10 minutes at 10°C (Eppendorf 5804 R, Germany) and stored at -40°C for biochemical analysis and analyzed within 12 hrs.

3.9.3 Haematological evaluation

Blood samples collected in heparinized test tubes at predetermined time intervals during course of experiment were used to evaluate parameters like haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC). The Hb, PCV, TLC and DLC were estimated using automated haematology analyzer (Mindray BC-2800 Vet) at Department of Veterinary Biochemistry and Biotechnology, Veterinary College, Anand Agricultural University, Anand.

3.9.4 Blood biochemical parameters

Serum from blood samples collected at predetermined time intervals was used to determine serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (AKP), serum lactate dehydrogenase (LDH), Serum creatinine, serum bilirubin (total), blood urea nitrogen and total protein. All the biochemical parameters were estimated using standard assay kits (Coral Clinical System, Goa, India) with the help of clinical serum biochemistry analyzer (BS-120 Chemistry Analyzer, Mindray) at Department of Veterinary Biochemistry, Veterinary College, Anand Agricultural University, Anand. Methods employed for determination of various haematological and biochemical indices have been presented in Table 3.5.

3.10 Statistical analysis

All the data have been presented as mean ± S.E. To evaluate the effect of tolfenamic acid and bioenhancer trikatu on pharmacokinetics of cefquinome in sheep were analyzed by two tails students' "t" test (paired) using Microsoft excel (version 2007). Similarly, data generated from the safety of cefquinome in combination with tolfenamic acid administration were analyzed by two tails students' "t" test (paired) using Microsoft excel (version 2007).

Table 3.5: Methods used for the determination of blood biochemical parameters

Sr. No.	Parameters	Method/References
1	Serum alanine aminotransferase (ALT) (IU/L)	UV kinetic method (Tietz, 1995)
2	Serum aspartate aminotransferase (AST) (IU/L)	UV kinetic method (Tietz, 1995)
3	Serum alkaline phosphatase (AKP) (IU/L)	Kinetic Method using p-nitrophenyl phosphate (Henry *et al.*, 1974)
4	Serum lactate dehydrogenase (LDH) (IU/L)	IFCC method (Tietz, 1987)
5	Serum Creatinine (mg/dL)	Alkaline picrate initial rate method (Henry *et al.*, 1974)
6	Serum Bilirubin (mg/dL)	Jendrassik & Grof method (Jendrassik and Grof, 1938)
7	Blood Urea Nitrogen (mg/dL)	Enzymatic UV-kinetic initial rate method (Talke and Schubert, 1965)
8	Total protein (gm/dL)	Biuret Method (Tietz, 1995)

CHAPTER - IV

RESULTS

The present study was undertaken to determine the effect of intramuscular administration of tolfenamic acid (2 mg/kg) and bioenhancer trikatu on pharmacokinetics of cefquinome following intramuscular administration (2 mg/kg) and safety of daily intramuscular administration of cefquinome (2 mg/kg) in combination with intramuscular administration of tolfenamic acid (2 mg/kg) for five days in sheep. The plasma samples collected for pharmacokinetic studies were assayed for cefquinome concentration using High Performance Liquid Chromatography (HPLC) procedure. The representative chromatograms of cefquinome (2.5 µg/mL) in mobile phase, blank plasma of sheep and cefquinome standard (2.5 µg/mL) in plasma of sheep have been shown in Figure 4.1. Calibration curves prepared from plasma standards were used to determine the concentration of cefquinome in plasma samples obtained from sheep for pharmacokinetic studies. Representative chromatograms of plasma samples collected after 1 h post intramuscular administration of cefquinome alone, in combination with tolfenamic acid and in trikatu pretreated sheep have been shown in Figure 4.1 Retention time of cefquinome was 3.22 ± 0.15 min. For safety study, blood and serum samples collected after daily intramuscular administration of cefquinome in combination with intramuscular administration of tolfenamic acid were processed for haematological and serum for biochemical analysis.

Chromatographs

Figure 4.1:

4.1 Plasma levels and pharmacokinetics of cefquinome following intramuscular administration in healthy sheep.

4.1.1 Plasma levels of cefquinome

The plasma levels of cefquinome as a function of time after its single intramuscular administration (2 mg/kg of body weight) in healthy sheep are depicted in Table 4.1, while semi logarithmic plot of the same has been presented as Figure 4.2.

Following intramuscular administration of the cefquinome, the drug concentration of 0.49 ± 0.01 μg/mL was observed at 0.083 h. The mean peak plasma drug concentration of 4.36 ± 0.10 μg/mL was achieved at 0.75 h which declined rapidly to 1.23 ± 0.03 μg/mL at 4 h. The drug concentration of 0.19 ± 0.01μg/mL in plasma was detected at 12 h. The drug was not detected in plasma samples collected after 18 h post intramuscular administration of cefquinome in sheep.

Table 4.1: Plasma concentrations (µg/mL) of cefquinome after single dose intramuscular administration (2 mg/kg) in healthy sheep

Time after drug administration (h)	Plasma concentration (µg/ml)						
	Sheep number						Mean ± S.E
	S1	S2	S3	S4	S5	S6	
0.083	0.46	0.50	0.48	0.48	0.50	0.50	0.49 ± 0.01
0.166	0.59	0.60	0.59	0.59	0.60	0.60	0.59 ± 0.01
0.25	0.78	0.80	0.79	0.78	0.80	0.80	0.79 ± 0.01
0.5	1.96	1.83	1.80	1.89	1.87	2.01	1.89 ± 0.01
0.75	3.93	4.54	4.46	4.21	4.54	4.50	4.36 ± 0.10
1	2.94	3.24	3.00	2.96	3.18	3.17	3.08 ± 0.05
2	2.22	2.27	2.26	2.23	2.27	2.27	2.25 ± 0.01
4	1.13	1.30	1.24	1.19	1.29	1.26	1.23 ± 0.03
8	0.54	0.54	0.54	0.54	0.54	0.54	0.54 ± 0.00
12	0.18	0.21	0.18	0.17	0.19	0.19	0.19 ± 0.01
18	0.15	0.12	0.14	0.10	0.14	0.12	0.13 ± 0.01
24	ND	ND	ND	ND	ND	ND	ND

* ND: Not Detected

semilogarithmic plot

Figure 4.2.

4.1.2 Pharmacokinetics of cefquinome

Various pharmacokinetic parameters calculated from plasma concentration – time profile after single dose intramuscular administration of cefquinome (2 mg/kg) in healthy sheep are summarized in Table 4.2.

Following intramuscular administration of the drug, the absorption rate constant (K_a) varied from 0.69 to 1.79 h^{-1} with an average of 1.27 ± 0.17 h^{-1}. The mean elimination rate constant (β) was 0.07 ± 0.01 h^{-1}. The absorption ($t_{½Ka}$) and elimination half-life ($t_{½β}$) were 0.61 ± 0.10 and 12.29 ± 2.62 h, respectively. The mean apparent volume of distribution ($Vd_{(area)}$), area under plasma drug concentration-time curve ($AUC_{(0 - ∞)}$) and area under first moment curve (AUMC) were 2.07 ± 0.36 L/kg, 16.65 ± 0.57 μg.h/mL and 157.05 ± 37.93 μg.h^2/mL, respectively. The mean value of total body clearance (Cl_B) of the drug was 0.12 ± 0.01 L/h/kg with mean residence time (MRT) of 9.14 ± 1.83 h.

Table 4.2: Pharmacokinetic parameters of cefquinome after single dose intramuscular administration (2 mg/kg) in healthy sheep

Pharmacokinetic parameter	Sheep Number							Mean ± S.E
	Unit	S1	S2	S3	S4	S5	S6	
A	µg/mL	2.43	2.99	2.94	2.36	3.25	2.90	2.81 ± 0.14
B	µg/mL	0.25	0.72	0.33	0.51	0.37	0.51	0.45 ± 0.07
K_a	h^{-1}	0.69	1.79	1.26	0.89	1.65	1.35	1.27 ± 0.17
α	h^{-1}	0.25	0.32	0.28	0.26	0.30	0.29	0.28 ± 0.01
β	h^{-1}	0.03	0.10	0.05	0.09	0.06	0.08	0.07 ± 0.01
$t_{½Ka}$	h	1.00	0.39	0.55	0.78	0.42	0.51	0.61 ± 0.10
$t_{½α}$	h	2.79	2.14	2.48	2.66	2.33	2.35	2.46 ± 0.10
$t_{½β}$	h	24.00	6.81	14.14	7.78	12.57	8.45	12.29 ± 2.62
C_{max}	µg/mL	3.93	4.54	4.46	4.21	4.54	4.50	4.36 ± 0.10
T_{max}	h	0.75	0.75	0.75	0.75	0.75	0.75	0.75 ± 0.01
$AUC_{(0-\infty)}$	µg.h/mL	18.97	15.88	17.05	15.00	17.10	15.92	16.65 ± 0.57
AUMC	µg.h^2/mL	335.04	93.54	168.14	90.61	151.68	103.31	157.05 ± 37.93
$Vd_{(area)}$	L/kg	3.65	1.24	2.39	1.50	2.12	1.53	2.07 ± 0.36
$Vd_{(ss)}$	L/kg	1.86	0.74	1.16	0.81	1.04	0.81	1.07 ± 0.17
$Cl_{(B)}$	L/h/kg	0.11	0.13	0.12	0.13	0.12	0.13	0.12 ± 0.01
MRT	h	17.67	5.89	9.86	6.04	8.87	6.49	9.14 ± 1.83

4.2 Plasma levels and pharmacokinetics of cefquinome following intramuscular administration in tolfenamic acid-treated sheep

4.2.1 Plasma levels of cefquinome

The plasma levels of cefquinome as a function of time following its single intramuscular administration (2 mg/kg of body weight) in tolfenamic acid -treated (2 mg/kg of body weight) sheep are depicted in Table 4.3, while semi logarithmic plot of the same have been presented as Figure 4.3

Following intramuscular administration of the cefquinome, the drug concentration of 0.51 ± 0.01 µg/mL was observed at 0.083 h. The mean peak plasma drug concentration of 4.73 ± 0.05 µg/mL was achieved at 0.75 h which declined rapidly to 1.37 ± 0.01 µg/mL at 4 h. The drug concentration of 0.15 ±0.01 µg/mL in plasma was detected at 18 h. The drug was not detected in plasma samples collected after 18 h post intramuscular administration of cefquinome in sheep.

4.2.2 Pharmacokinetics of cefquinome in presence of tolfenamic acid

Various pharmacokinetic parameters calculated from plasma concentration – time profile after single dose intramuscular administration of cefquinome (2 mg/kg) in tolfenamic acid -treated (2 mg/kg, IM) sheep are summarized in Table 4.4.

Following intramuscular administration of the drug in tolfenamic acid treated sheep, the absorption rate constant (K_a) varied from 2.34 to 3.54 h^{-1} with an average of 2.75 ± 0.17 h^{-1}. The mean elimination rate constant (β) was 0.08 ± 0.01 h^{-1}. The absorption ($t_{½Ka}$) and elimination half-life ($t_{½β}$) were 0.26 ± 0.01 and 9.00 ± 0.51 h, respectively.

Table 4.3: Plasma concentrations (µg/mL) of cefquinome following intramuscular administration (2 mg/kg) in tolfenamic acid -treated (2 mg/kg) sheep

Time after drug administration (h)	Plasma concentration (µg/ml)						
	Sheep number						Mean ± S.E
	S1	S2	S3	S4	S5	S6	
0.083	0.52	0.51	0.51	0.52	0.51	0.52	0.51 ± 0.01
0.166	0.61	0.60	0.60	0.61	0.60	0.62	0.61 ± 0.01
0.25	0.89	0.81	0.81	0.89	0.82	0.90	0.85 ± 0.02
0.5	2.03	2.14	2.21	2.16	2.20	2.09	2.14 ± 0.03
0.75	4.79	4.68	4.55	4.77	4.76	4.87	4.73 ± 0.05
1	3.44	3.31	3.24	3.41	3.39	3.45	3.37 ± 0.03
2	2.41	2.36	2.27	2.40	2.39	2.41	2.38 ± 0.02
4	1.40	1.35	1.33	1.38	1.36	1.40	1.37 ± 0.01
8	0.55	0.55	0.55	0.55	0.55	0.56	0.55 ± 0.01
12	0.24	0.24	0.21	0.24	0.24	0.26	0.24 ± 0.01
18	0.16	0.16	0.14	0.15	0.15	0.14	0.15 ± 0.01
24	ND	ND	ND	ND	ND	ND	ND

* ND: Not Detected

Figure 4.3

Semilogarithmic plot

The mean apparent volume of distribution ($Vd_{(area)}$), area under plasma drug concentration-time curve ($AUC_{(0 - \infty)}$) and area under first moment curve (AUMC) were 1.48 ± 0.08 L/kg, 17.52 ± 0.14 µg.h/mL and 127.55 ± 5.24 $\mu g.h^2/mL$, respectively. The mean value of total body clearance (Cl_B) of the drug was 0.11 ± 0.01 L/h/kg with mean residence time (MRT) of 7.27 ± 0.27 h.

4.3 Effect on plasma levels and pharmacokinetics of cefquinome (2 mg/kg body weight) following intramuscular administration in sheep pretreated with trikatu.

The plasma levels of cefquinome as a function of time following its single intramuscular administration (2 mg/kg body weight) in trikatu pretreated sheep (7 days before cefquinome administration) are depicted in Table 4.5., while semi logarithmic plot of the same has been presented as Figure 4.4

Following intramuscular administration of the cefquinome in trikatu pretreated sheep, the drug concentration of 0.52 ± 0.01µg/mL was observed at 0.083 h. The mean peak plasma drug concentration of 5.23 ± 0.03 µg/mL was achieved at 0.75 h which declined rapidly to 1.51 ± 0.03 µg/mL at 4 h. Thereafter, the drug concentration in plasma diminished gradually and was detectable up to 18 h. The drug concentration of 0.16 ± 0.01 µg/mL in plasma was detected at 18 h and beyond this drug was not detected in plasma samples collected after 18 h post intramuscular administration of cefquinome in trikatu pretreated sheep.

Table 4.4: Pharmacokinetic parameters of cefquinome after single dose intramuscular administration (2 mg/kg) in tolfenamic acid-treated sheep

Pharmacokinetic parameter	Sheep Number							Mean ± SE
	Unit	S1	S2	S3	S4	S5	S6	
A	µg/mL	4.12	3.77	3.45	3.88	3.75	3.99	3.83 ± 0.09
B	µg/mL	0.58	0.54	0.49	0.66	0.58	0.88	0.62 ± 0.06
K_a	h^{-1}	2.45	3.54	2.66	2.83	2.68	2.34	2.75 ± 0.17
	h^{-1}	0.36	0.35	0.32	0.36	0.35	0.40	0.36 ± 0.01
	h^{-1}	0.07	0.07	0.07	0.08	0.07	0.10	0.08 ± 0.01
$t_{½Ka}$	H	0.28	0.20	0.26	0.24	0.26	0.30	0.26 ± 0.01
$t_{½\alpha}$	H	1.90	2.00	2.16	1.91	1.98	1.75	1.95 ± 0.06
$t_{½\beta}$	H	9.57	10.12	9.84	8.35	9.34	6.79	9.00 ± 0.51
C_{max}	µg/mL	4.79	4.68	4.55	4.77	4.76	4.87	4.73 ± 0.05
T_{max}	H	0.75	0.75	0.75	0.75	0.75	0.75	0.75 ± 0.01
$AUC_{(0-\infty)}$	µg.h/mL	17.99	17.75	17.01	17.47	17.65	17.27	17.52 ± 0.14
AUMC	$µg.h^2/mL$	137.57	141.70	127.63	120.54	131.69	106.14	127.55 ± 5.24
$Vd_{(area)}$	L/kg	1.54	1.65	1.67	1.38	1.53	1.13	1.48 ± 0.08
$Vd_{(ss)}$	L/kg	0.85	0.90	0.88	0.79	0.85	0.71	0.83 ± 0.03
$Cl_{(B)}$	L/h/kg	0.11	0.11	0.12	0.11	0.11	0.12	0.11 ± 0.01
MRT	h	7.65	7.98	7.51	6.90	7.46	6.15	7.27 ± 0.27

Table 4.5: Plasma concentrations (µg/mL) of cefquinome following single dose intramuscular administration (2 mg/kg body weight.) in trikatu pretreated sheep.

Time after drug administration (h)	Plasma concentration (µg/ml)						
	Sheep number						Mean ± S.E
	S1	S2	S3	S4	S5	S6	
0.083	0.52	0.53	0.44	0.53	0.54	0.53	0.52 ± 0.01
0.166	0.62	0.63	0.63	0.63	0.63	0.62	0.63 ± 0.01
0.25	0.91	1.00	1.03	1.01	1.02	0.94	0.98 ± 0.02
0.5	2.47	2.55	2.61	2.75	2.62	2.73	2.62 ± 0.04
0.75	5.08	5.11	5.52	5.14	5.48	5.08	5.23 ± 0.08
1	3.63	3.76	3.91	3.78	3.88	3.65	3.77 ± 0.05
2	2.42	2.43	2.44	2.43	2.43	2.43	2.43 ± 0.01
4	1.42	1.47	1.60	1.55	1.59	1.44	1.51 ± 0.03
8	0.56	0.56	0.59	0.57	0.58	0.56	0.57 ± 0.01
12	0.24	0.26	0.31	0.29	0.30	0.25	0.28 ± 0.01
18	0.17	0.16	0.16	0.16	0.16	0.16	0.16 ± 0.01
24	ND	ND	ND	ND	ND	ND	ND

* ND: Not Detected

Figure 4.4: Semi logarithmic

4.3.2 Pharmacokinetics of cefquinome

Various pharmacokinetic parameters calculated from plasma concentration – time profile after single dose intramuscular administration of cefquinome (2 mg/kg) in trikatu pretreated sheep are summarized in Table 4.6.

Following intramuscular administration of the drug, the absorption rate constant (K_a) varied from 1.22 to 2.12 h^{-1} with an average of 1.63 ± 0.16 h^{-1}. The mean elimination rate constant (β) was 0.09 ± 0.01 h^{-1}. The absorption ($t_{½Ka}$) and elimination half-life ($t_{½β}$) were 0.45 ± 0.04 and 8.33 ± 0.79 h, respectively. The mean apparent volume of distribution ($Vd_{(area)}$), area under plasma drug concentration-time curve ($AUC_{(0 - ∞)}$) and area under first moment curve (AUMC) were 1.27 ± 0.12 L/kg, 18.89 ± 0.06 μg.h/mL and 133.24 ± 7.28 μg.h^2/mL, respectively. The mean value of total body clearance (Cl_B) of the drug was 0.11 ± 0.01 L/h/kg with mean residence time (MRT) of 7.06 ± 0.39 h.

Table 4.6: Pharmacokinetic parameters of cefquinome following single dose intramuscular administration (2 mg/kg) in trikatu pretreated sheep

Pharmacokinetic parameter	Sheep Number							Mean ± SE
	Unit	S1	S2	S3	S4	S5	S6	
A	µg/mL	4.20	4.74	7.19	5.80	6.24	4.37	5.42 ± 0.48
B	µg/mL	0.51	0.68	1.20	0.94	0.99	0.59	0.82 ± 0.11
K_a	h^{-1}	2.12	1.82	1.22	1.36	1.29	1.99	1.63 ± 0.16
	h^{-1}	0.35	0.39	0.54	0.46	0.48	0.37	0.43 ± 0.03
	h^{-1}	0.06	0.08	0.11	0.10	0.10	0.07	0.09 ± 0.01
$t_{½Ka}$	h	0.33	0.38	0.57	0.51	0.54	0.35	0.45 ± 0.04
$t_{½\alpha}$	h	1.96	1.76	1.29	1.50	1.45	1.87	1.64 ± 0.11
$t_{½\beta}$	h	11.21	8.75	6.20	7.11	6.91	9.80	8.33 ± 0.79
C_{max}	µg/mL	5.08	5.11	5.52	5.14	5.48	5.08	5.23 ± 0.08
T_{max}	h	0.75	0.75	0.75	0.75	0.75	0.75	0.75 ± 0.01
$AUC_{(0-\infty)}$	µg.h/mL	18.97	18.72	19.06	18.81	19.05	18.74	18.89 ± 0.06
AUMC	µg.h^2/mL	162.42	134.47	115.73	121.50	120.57	144.76	133.24 ± 7.28
$Vd_{(area)}$	L/kg	1.71	1.35	0.94	1.09	1.05	1.51	1.27 ± 0.12
$Vd_{(ss)}$	L/kg	0.90	0.77	0.64	0.69	0.66	0.82	0.75 ± 0.04
$Cl_{(B)}$	L/h/kg	0.11	0.11	0.10	0.11	0.10	0.13	0.11 ± 0.01
MRT	h	8.56	7.18	6.07	6.46	6.33	7.72	7.06 ± 0.39

4.4 Effect of intramuscular administration of tolfenamic acid (2 mg/kg) and trikatu on plasma concentration of cefquinome following its intramuscular administration (2 mg/kg) in healthy sheep

The mean plasma concentration - time data following intramuscular administration of cefquinome (2 mg/kg) in normal, tolfenamic acid - treated (2 mg/kg) and trikatu pretreated sheep are presented in Table 4.7, while semi logarithmic plot of the same has been presented as Figure 4.5.

Following intramuscular administration of cefquinome in tolfenamic acid - treated sheep, peak plasma concentration of 4.73 ± 0.05 μg/mL observed at 0.75 h was significantly higher than normal sheep. The plasma drug concentrations were significantly higher from 0.0835 to 18 h post administration of drug in tolfenamic acid-treated sheep compared to normal as shown in Table 4.7. Last observed plasma drug concentration was also significantly higher in tolfenamic acid-treated sheep than normal. Following intramuscular administration of cefquinome in trikatu pretreated sheep, peak plasma concentration of 5.23 ± 0.08 μg/mL observed at 0.75 h was significantly higher than cefquinome alone treated healthy sheep. Plasma drug concentrations observed from 0.0835 to 18 h in trikatu pretreated sheep were significantly higher than healthy sheep.

Clinical examination of all animals before and after each study did not reveal any abnormalities. No local or systemic adverse reaction/s to cefquinome occurred after intramuscular administration in sheep.

Table 4.7: Comparison of plasma concentrations (µg/mL) of cefquinome after intramuscular administration (2 mg/kg) in normal, Tolfenamic acid-treated (2 mg/kg) and in trikatu pretreated sheep (n=6)

Time after drug administration (h)	Sheep		
	Normal / Healthy	Tolfenamic acid-treated	Trikatu pretreated
0.083	0.49 ± 0.01	0.51 ± 0.01*	0.52 ± 0.01
0.166	0.59 ± 0.01	0.61 ± 0.01**	0.63 ± 0.01**
0.25	0.79 ± 0.01	0.85 ± 0.02*	0.98 ± 0.02**
0.5	1.89 ± 0.01	2.14 ± 0.03**	2.62 ± 0.04**
0.75	4.36 ± 0.10	4.73 ± 0.05*	5.23 ± 0.08**
1	3.08 ± 0.05	3.37 ± 0.03**	3.77 ± 0.05**
2	2.25 ± 0.01	2.38 ± 0.02**	2.43 ± 0.01**
4	1.23 ± 0.03	1.37 ± 0.01**	1.51 ± 0.03**
8	0.54 ± 0.00	0.55 ± 0.01**	0.57 ± 0.01**
12	0.19 ± 0.01	0.24 ± 0.01**	0.28 ± 0.01**
18	0.13 ± 0.01	0.15 ± 0.01*	0.16 ± 0.01**
24	ND	ND	ND

ND: Not Detected; *Significant at $p<0.05$, **highly significant at $p<0.01$ when compared with respective values of healthy sheep

Figure 4.5: Semilogarithmic plot of

CEQ

+

TA

+

TKT

4.5 Effect of intramuscular administration of tolfenamic acid (2 mg/kg) and trikatu on pharmacokinetics of cefquinome following its intramuscular administration (2 mg/kg) in healthy sheep

Comparison of pharmacokinetic parameters (Mean ± SE) of cefquinome after intramuscular administration (2 mg/kg) in normal, tolfenamic acid-treated (2 mg/kg) and trikatu pretreated sheep are elicited in Table 4.8.

Following intramuscular administration of cefquinome in tolfenamic acid-treated sheep, significant decrease in $t_{½}$ and $t_{½Ka}$ where as significant increase in A, K_a, α, and C_{max} were observed as compared to respective pharmacokinetic parameters of cefquinome in healthy sheep. Following intramuscular administration of cefquinome in trikatu pretreated sheep, significant decrease in $t_{½\alpha}$ where as significant increase in A, B, α, C_{max}, and $AUC_{(0-)}$ were observed as compared to respective pharmacokinetic parameters of cefquinome in healthy sheep.

Table 4.8: Comparison of pharmacokinetic parameters (Mean ± SE) of cefquinome after intramuscular administration (2 mg/kg) in normal, tolfenamic acid-treated (2 mg/kg) and trikatu pretreated sheep (n=6)

Pharmacokinetic parameter	Unit	Sheep		
		Normal / Healthy	Tolfenamic acid-treated	Trikatu pretreated
A	µg/mL	2.81 ± 0.14	3.83 ± 0.09**	5.42 ± 0.48**
B	µg/mL	0.45 ± 0.07	0.62 ± 0.06	0.82 ± 0.11*
K_a	h^{-1}	1.27 ± 0.17	2.75 ± 0.17**	1.63 ± 0.16
α	h^{-1}	0.28 ± 0.01	0.36 ± 0.01**	0.43 ± 0.03**
β	h^{-1}	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
$t_{½Ka}$	h	0.61 ± 0.10	0.26 ± 0.01**	0.45 ± 0.04
$t_{½α}$	h	2.46 ± 0.10	1.95 ± 0.06**	1.64 ± 0.11**
$t_{½β}$	h	12.29 ± 2.62	9.00 ± 0.51	8.33 ± 0.79
C_{max}	µg/mL	4.36 ± 0.10	4.73 ± 0.05*	5.23 ± 0.08**
T_{max}	h	0.75 ± 0.01	0.75 ± 0.01	0.75 ± 0.01
$AUC_{(0-\infty)}$	µg.h/mL	16.65 ± 0.57	17.52 ± 0.14	18.89 ± 0.06**
AUMC	µg.h²/mL	157.05 ± 37.93	127.55 ± 5.24	133.24 ± 7.28
$Vd_{(area)}$	L/kg	2.07 ± 0.36	1.48 ± 0.08	1.27 ± 0.12
$Vd_{(ss)}$	L/kg	1.07 ± 0.17	0.83 ± 0.03	0.75 ± 0.04
$Cl_{(B)}$	L/h/kg	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
MRT	h	9.14 ± 1.83	7.27 ± 0.27	7.06 ± 0.39

*Significant at $p<0.05$, **Highly significant at $p<0.01$ when compared with respective values of healthy sheep

4.6 Studies on safety assessment of the cefquinome in combination with tolfenamic acid in healthy sheep

4.6.1 Haematological parameters

Values of haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count (neutrophil, lymphocyte, basophil, eosinophil and monocyte) following daily intramuscular administration of cefquinome (2 mg/kg) in combination with intramuscular administration of tolfenamic acid (2 mg/kg) for 5 days in healthy sheep are presented in Tables 4.9 to 4.17 and the same have been depicted as Figures 4.6 to 4.10.

No significant alterations ($p < 0.05$) in haemoglobin level, pack cell volume, total leukocyte count and differential leukocyte count have been observed following daily intramuscular administration of cefquinome in combination with tolfenamic acid for 5 days in healthy sheep.

4.6.2 Blood biochemical parameters

The values of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (AKP), serum lactate dehydrogenase (LDH), serum bilirubin (total), serum creatinine, blood urea nitrogen and total protein following daily intramuscular administration of cefquinome (2 mg/kg) in combination with intramuscular administration of tolfenamic acid (2 mg/kg) for 5 days in healthy sheep are presented in Tables 4.18 to 4.25 and the same are depicted as Figures 4.11 to 4.18, respectively. No significant alterations ($p < 0.05$) in all blood biochemical parameters have been observed following daily intramuscular administration of cefquinome in combination with tolfenamic acid for 5 days in healthy sheep.

Table 4.9: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood Haemoglobin level (g/dL) in sheep

Time of Treatment (Days)	Haemoglobin (g/dL)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	10.00	9.50	9.60	10.50	11.30	10.70	10.27 ± 0.28
1	9.60	9.35	9.50	10.35	11.35	10.61	10.13 ± 0.32
2	9.80	9.70	9.65	10.20	11.10	10.20	10.11 ± 0.22
3	10.10	9.20	9.82	10.60	11.45	11.01	10.36 ± 0.34
4	9.60	9.50	9.70	10.75	10.90	11.21	10.28 ± 0.31
5	9.50	9.25	9.65	10.40	11.40	10.85	10.18 ± 0.35

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.10: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood PCV level (per cent) in sheep

Time of Treatment (Days)	Packed Cell Volume (per cent)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	29.4	29.8	28.6	30.0	28.9	29.9	29.43 ± 0.23
1	32.0	30.5	29.4	30.5	29.0	30.4	30.30 ± 0.43
2	31.4	31.3	29.3	30.7	30.9	28.1	30.28 ± 0.53
3	26.4	27.5	28.8	29.2	30.4	28.4	28.45 ± 0.57
4	28.5	30.6	28.1	30.6	31.1	27.8	29.45 ± 0.60
5	30.1	31.3	28.5	29.6	29.5	30.2	29.87 ± 0.38

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.11: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood Total Erythrocyte Count (× 10^6/µl) in sheep

Time of Treatment (Days)	Total Erythrocyte Count (× 10^6/µl)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	9.2	12.8	13.5	8.7	9.3	13.1	11.10 ± 0.92
1	10.95	11.24	10.56	11.04	10.9	10.86	10.93 ± 0.09
2	10.93	10.57	12.7	10.61	10.86	11.06	11.12 ± 0.32
3	11.03	11	11.15	11.25	11.64	10.9	11.16 ± 0.11
4	10.9	11.27	11.01	11.1	10.8	10.76	10.97 ± 0.08
5	9.8	12.6	11.8	9.8	10.1	11.65	10.96 ± 0.49

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.12: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood Total Leucocyte Count (× 10^3/cmm) in sheep

Time of Treatment (Days)	Total Leucocyte Count (× 10^3/cubic milliliter)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	7.1	7.3	8.9	7.1	8.6	8.5	7.92 ± 0.34
1	7.4	7.3	9.9	6.7	8.9	9.8	8.33 ± 0.56
2	7.6	7.5	9.6	6.8	9.2	9.7	8.40 ± 0.51
3	7.5	7.4	9.6	6.8	8.8	9.7	8.30 ± 0.50
4	9.2	12.8	8.4	8.7	9.3	13.1	10.25 ± 0.87
5	7.8	7.5	8.5	8.3	8.2	12.3	8.77 ± 0.72

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.13: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on Neutrophils count (per cent) in sheep

Time of Treatment (Days)	Neutrophils Count (per cent)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	38	38	38	39	39	36	38.00 ± 0.45
1	37	39	40	28	41	41	37.67 ± 2.03
2	37	41	41	36	37	40	38.67 ± 0.92
3	41	37	39	41	40	39	39.50 ± 0.62
4	39	35	42	40	39	37	38.67 ± 0.99
5	42	39	37	38	40	39	39.17 ± 0.70

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.14: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on Eosinophils count (per cent) in sheep

Time of Treatment (Days)	Eosinophils Count (per cent)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	2	3	2	4	3	2	2.67 ± 0.33
1	3	3	3	2	3	3	2.83 ± 0.17
2	3	2	2	2	1	2	2.00 ± 0.26
3	2	2	3	4	2	3	2.67 ± 0.33
4	2	3	2	3	3	2	2.50 ± 0.22
5	4	1	1	2	4	2	2.33 ± 0.56

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.15: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on Basophils count (per cent) in sheep

Time of Treatment (Days)	Basophils Count (per cent)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	1	1	1	1	1	0	0.83 ± 0.17
1	0	0	1	0	0	1	0.33 ± 0.21
2	1	0	0	1	2	0	0.67 ± 0.33
3	0	1	1	1	0	0	0.50 ± 0.22
4	1	0	1	0	1	1	0.67 ± 0.21
5	1	1	1	1	0	1	0.83 ± 0.17

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.16: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on monocytes count (per cent) in sheep

Time of Treatment (Days)	Monocytes Count (per cent)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	2	1	2	2	1	2	1.67 ± 0.21
1	3	3	1	2	2	1	2.00 ± 0.37
2	1	3	2	1	1	1	1.50 ± 0.34
3	2	4	3	3	1	2	2.50 ± 0.43
4	3	3	1	4	3	3	2.83 ± 0.40
5	2	2	2	3	2	2	2.17 ± 0.17

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.17: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on Lymphocytes count (per cent) in sheep

Time of Treatment (Days)	Lymphocytes Count (per cent)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	54	57	56	57	57	56	56.17 ± 0.48
1	52	56	57	58	54	58	55.83 ± 0.98
2	55	56	57	54	60	56	56.33 ± 0.84
3	52	55	53	57	54	57	54.67 ± 0.84
4	53	54	55	58	55	58	55.50 ± 0.85
5	56	55	53	56	57	57	55.67 ± 0.61

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.18: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum Alkaline Phosphate level (IU/L) in sheep

Time of Treatment (Days)	Alkaline Phosphate Level (IU/L)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	178.7	93.92	128.03	125.6	103.69	99.61	121.59 ± 12.76
1	157.98	99.07	137.76	117.45	105.9	90.97	118.19 ± 10.37
2	180.91	90.97	120.25	122.8	103.45	93.92	118.72 ± 13.55
3	155.11	101.05	139.92	118.6	102.55	101.05	119.71 ± 9.41
4	154.76	90.41	120.1	116.75	101.67	95.61	113.22 ± 9.57
5	179.25	95.61	119.5	110.45	101.3	90.41	116.09 ± 13.33

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.19: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum AST level (IU/L) in sheep

Time of Treatment (Days)	AST Level (IU/L)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	96.652	91.52	88.77	77.12	89.24	89.06	88.73 ± 2.62
1	89.69	89.35	95.45	74.56	88.32	83.64	86.84 ± 2.90
2	92.36	95.45	90.12	89.23	85.03	84.58	89.46 ± 1.71
3	95.78	92.37	86.65	85.78	83.41	83.74	87.96 ± 2.05
4	97.1	89.9	91.75	76.21	91.17	92.14	89.71 ± 2.88
5	95.49	84.67	92.64	84.65	87.78	87.19	88.74 ± 1.80

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.20: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum ALT level (IU/L) in sheep

Time of Treatment (Days)	ALT Level (IU/L)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	28.47	35.41	27.46	30.43	45.6	26.35	32.29 ± 2.96
1	25.45	24.76	31.38	29.41	44.6	27.15	30.46 ± 3.00
2	24.75	27.08	35.23	28.38	32.46	24.93	28.80 ± 1.72
3	25.06	26.98	30.46	28.16	32.416	27.32	28.40 ± 1.08
4	25.29	24.87	29.16	26.65	40.35	29.13	29.24 ± 2.34
5	24.14	24.32	28.65	32.19	46.8	24.98	30.18 ± 3.56

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.21: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum LDH level (IU/L) in sheep

Time of Treatment (Days)	LDH Level (IU/L)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	339.94	336.22	332.20	336.02	328.45	331.74	334.10 ± 1.67
1	334.54	335.20	334.60	330.54	327.15	330.90	332.85 ± 1.29
2	338.76	336.14	331.45	325.87	324.15	325.65	330.34 ± 2.49
3	335.04	320.65	322.63	334.60	327.34	317.64	326.08 ± 2.98
4	334.48	333.29	327.30	337.65	332.80	333.53	333.17 ± 1.37
5	339.75	329.24	329.27	335.57	330.41	335.57	333.30 ± 1.76

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.22: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum total bilirubin level (mg/dl) in sheep

Time of Treatment (Days)	Total Bilirubin Level (mg/dl)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	0.21	0.1	0.15	0.20	0.26	0.12	0.17 ± 0.03
1	0.2	0.17	0.10	0.12	0.17	0.2	0.16 ± 0.02
2	0.25	0.2	0.09	0.19	0.23	0.16	0.19 ± 0.02
3	0.26	0	0.18	0.10	0.14	0.13	0.14 ± 0.04
4	0.25	0.11	0.22	0.16	0.19	0.15	0.18 ± 0.02
5	0.12	0.14	0.09	0.24	0.21	0.19	0.17 ± 0.02

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.23: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum creatinine level (mg/dl) in sheep

Time of Treatment (Days)	Creatinine Level (mg/dl)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	1.45	1.25	1.66	1.20	1.25	1.23	1.34 ± 0.07
1	1.46	1.24	1.88	1.23	1.09	1.26	1.36 ± 0.11
2	1.33	1.31	1.47	1.10	1.11	1.22	1.26 ± 0.06
3	1.42	1.23	1.46	1.26	1.21	1.20	1.30 ± 0.05
4	1.43	1.24	1.86	1.18	1.2	1.21	1.35 ± 0.11
5	1.39	1.26	1.76	1.13	1.04	1.12	1.28 ± 0.11

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.24: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on BUN level (mg/dl) in sheep

Time of Treatment (Days)	Blood Urea Nitrogen (mg/dl)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	11.9	14.30	15.2	15.15	18.24	16.06	15.14 ± 0.85
1	11.5	18.62	14.58	19.65	16.94	14.54	15.97 ± 1.23
2	14.25	16.35	12.45	17.46	17.32	17.40	15.87 ± 0.85
3	14.98	15.20	15.71	15.79	18.72	16.23	16.11 ± 0.55
4	16.98	16.90	16.014	16.37	16.79	14.73	16.30 ± 0.35
5	17.21	19.40	14.55	18.85	17.67	12.57	16.71 ± 1.08

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

Table 4.25: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum total protein level (g/dL) in sheep

Time of Treatment (Days)	Total Protein Level (g/dL)						
	Sheep number						Mean ± SEM (n=6)
	S1	S2	S3	S4	S5	S6	
0	7.12	6.48	6.30	6.38	6.35	7.22	6.64 ± 0.17
1	6.79	6.98	6.42	7.41	7.85	6.92	7.06 ± 0.20
2	6.15	6.59	6.91	6.89	6.95	7.35	6.81 ± 0.16
3	7.59	7.59	6.10	6.79	7.59	7.07	7.12 ± 0.25
4	7.17	6.45	7.30	7.63	6.77	7.25	7.10 ± 0.17
5	7.20	7.62	6.10	6.51	6.36	6.59	6.73 ± 0.23

All values in treatment groups are not significantly different ($p<0.05$) when compared to control (0 h)

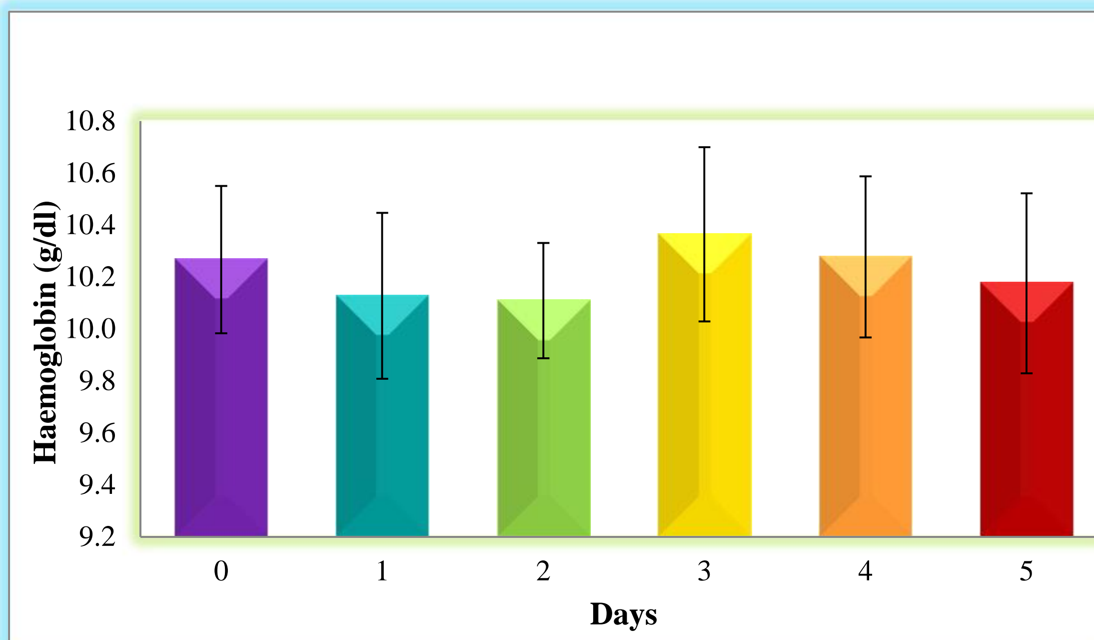

Figure 4.6: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood haemoglobin level (g/dL) in sheep.

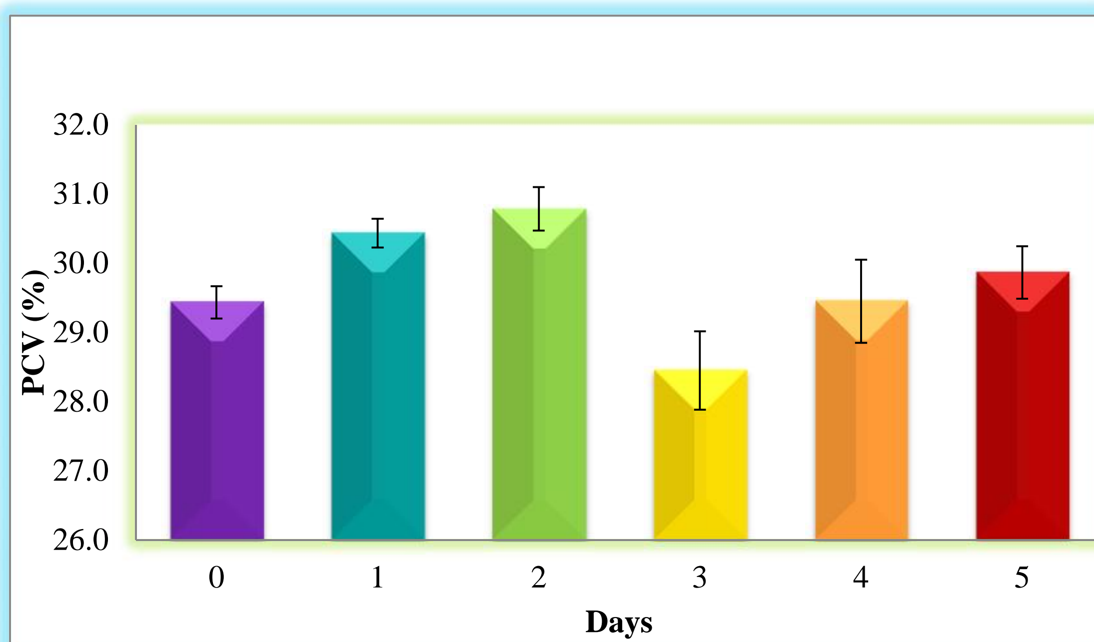

Figure 4.7: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood PCV level (per cent) in sheep.

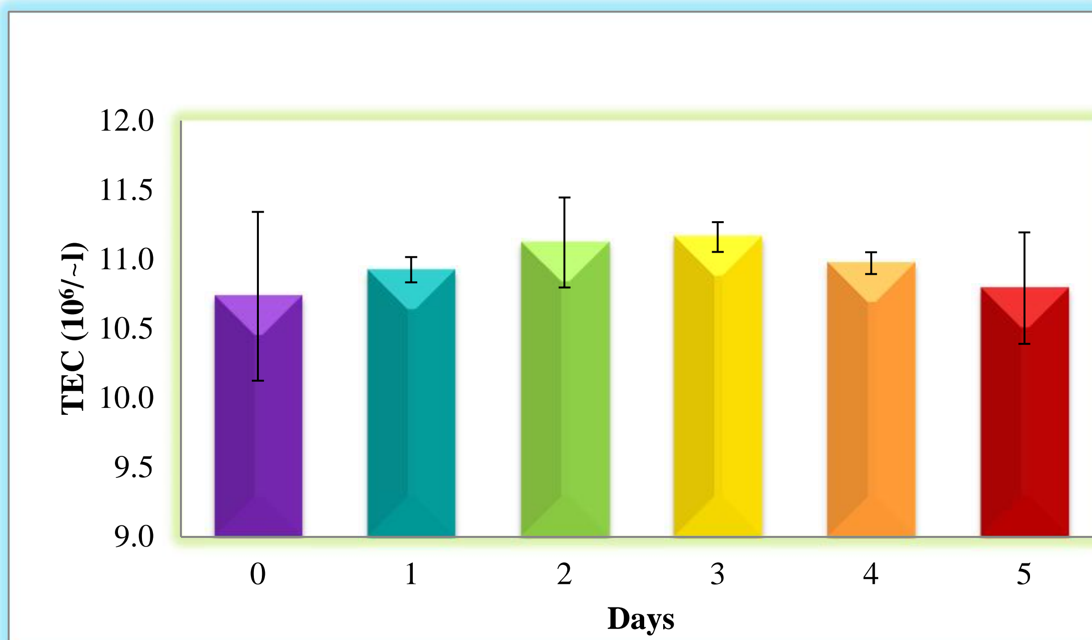

Figure 4.8: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood Total erythrocyte Count ($\times 10^6/\mu l$) in sheep.

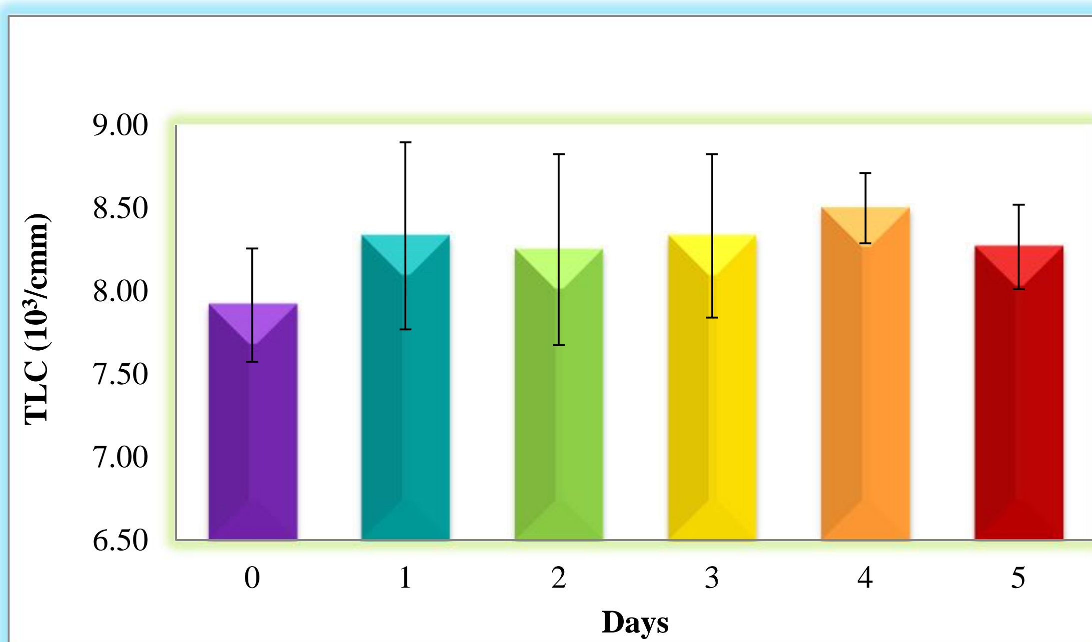

Figure 4.9: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on blood Total leucocyte Count ($\times 10^3$/cmm) in sheep.

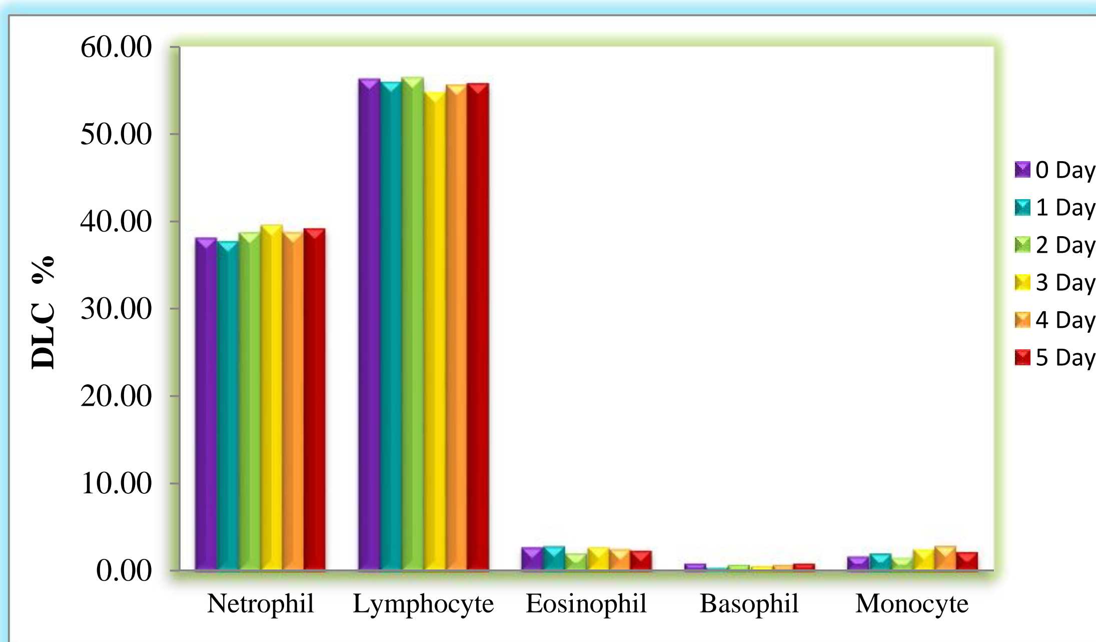

Figure 4.10: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on differential leukocyte count (per cent) in sheep.

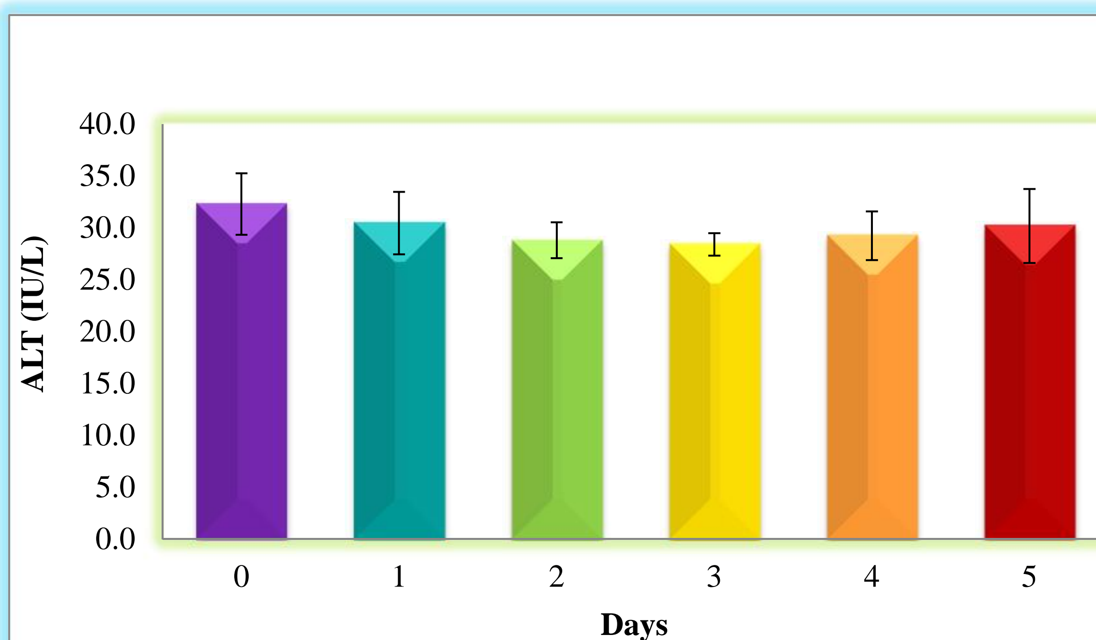

Figure 4.11: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum ALT level (IU/L) in sheep.

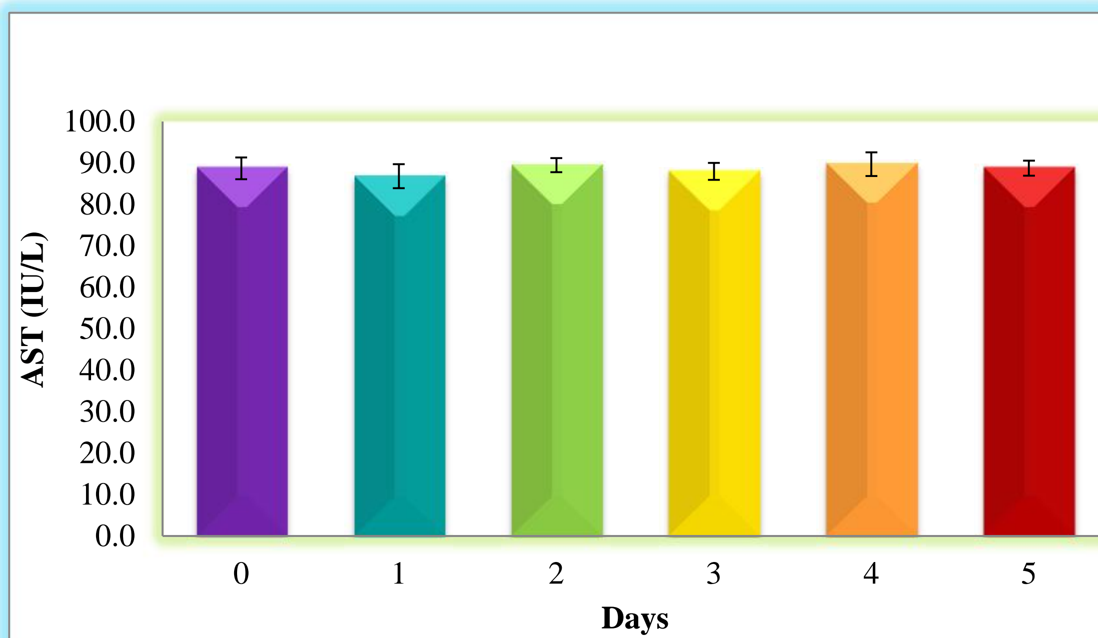

Figure 4.12: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum AST level (IU/L) in sheep.

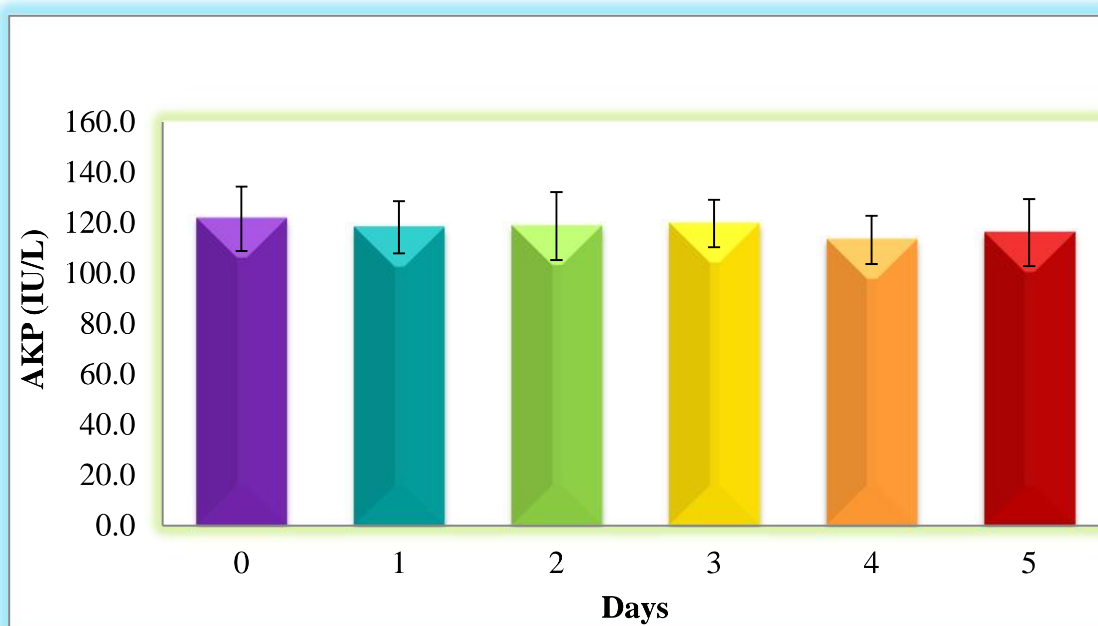

Figure 4.13: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum alkaline phosphate level (IU/L) in sheep.

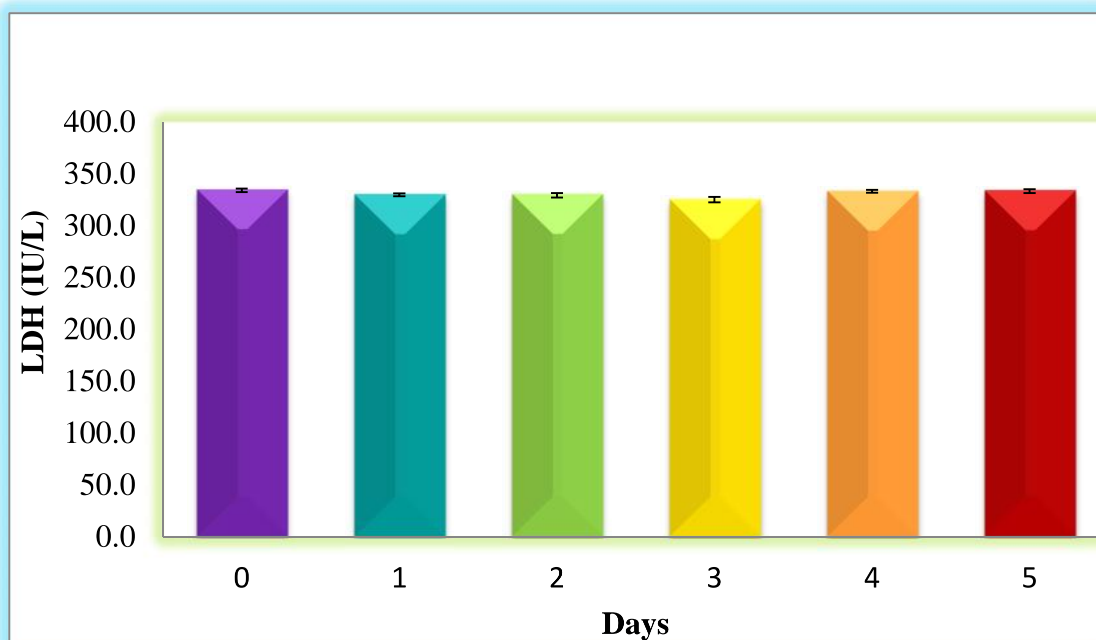

Figure 4.14: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum LDH level (IU/L) in sheep.

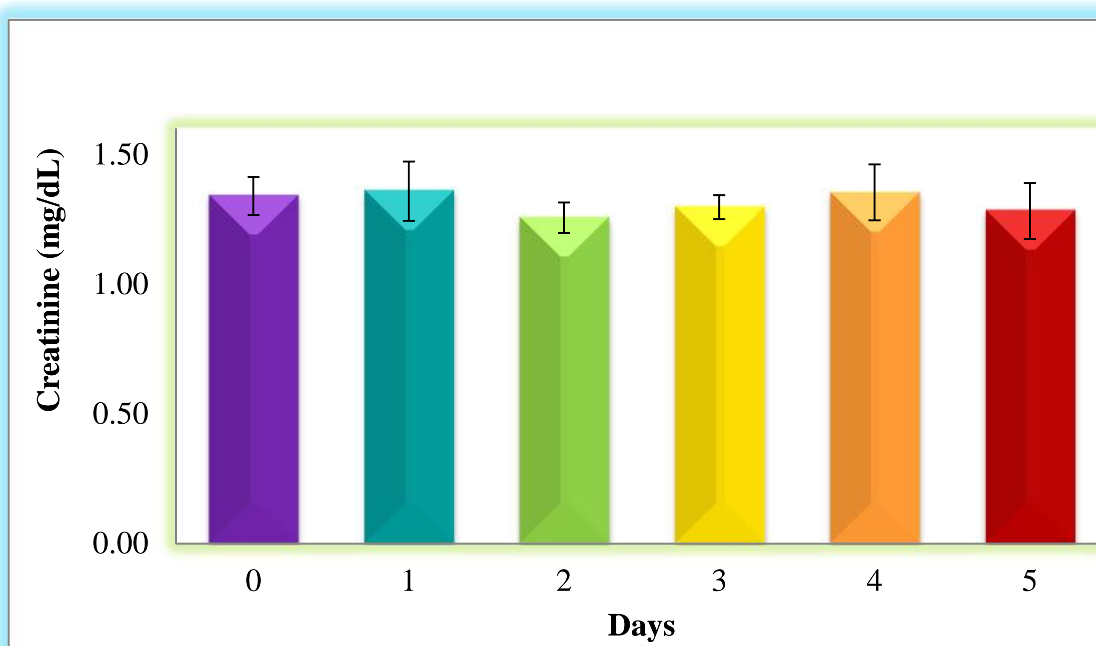

Figure 4.15: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum creatinine level (mg/dL) in sheep.

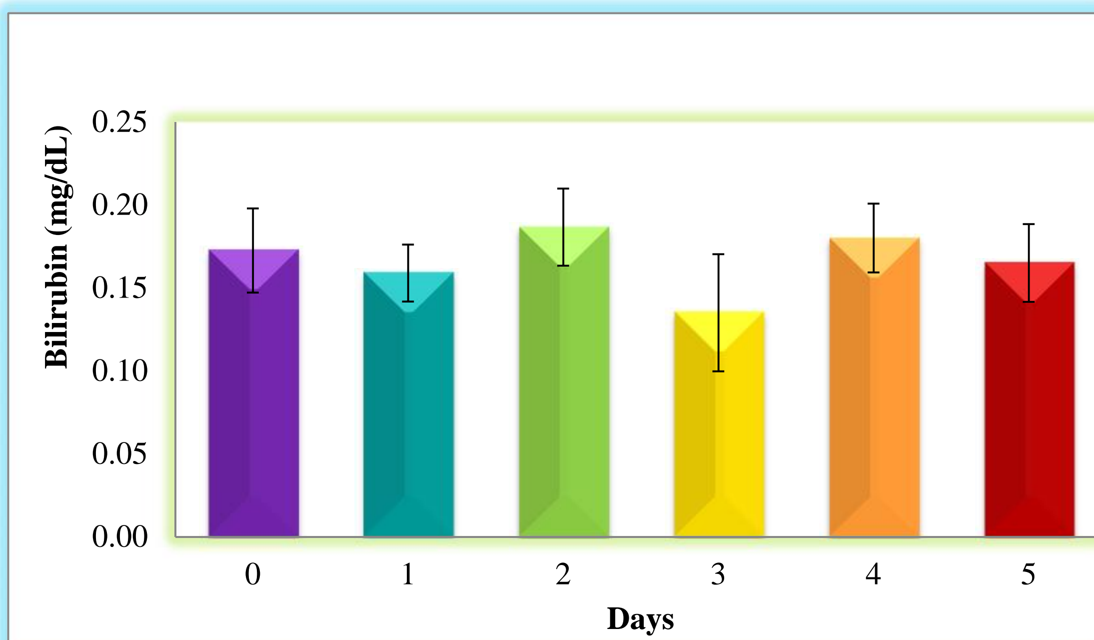

Figure 4.16: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum total bilirubin level (mg/dL) in sheep.

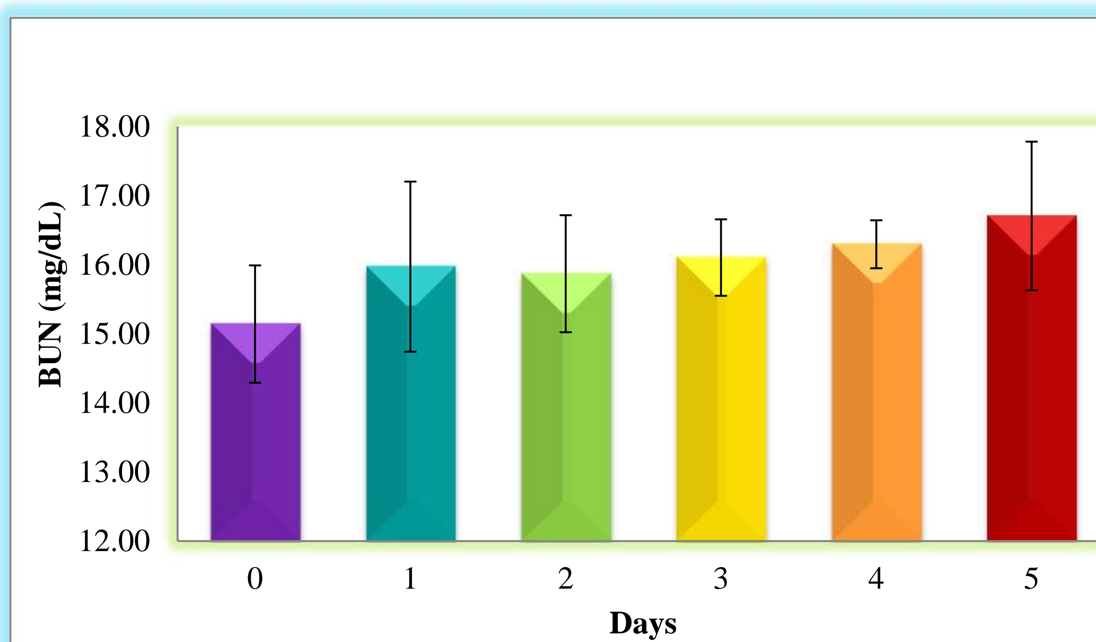

Figure 4.17: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on BUN level (mg/dL) in sheep.

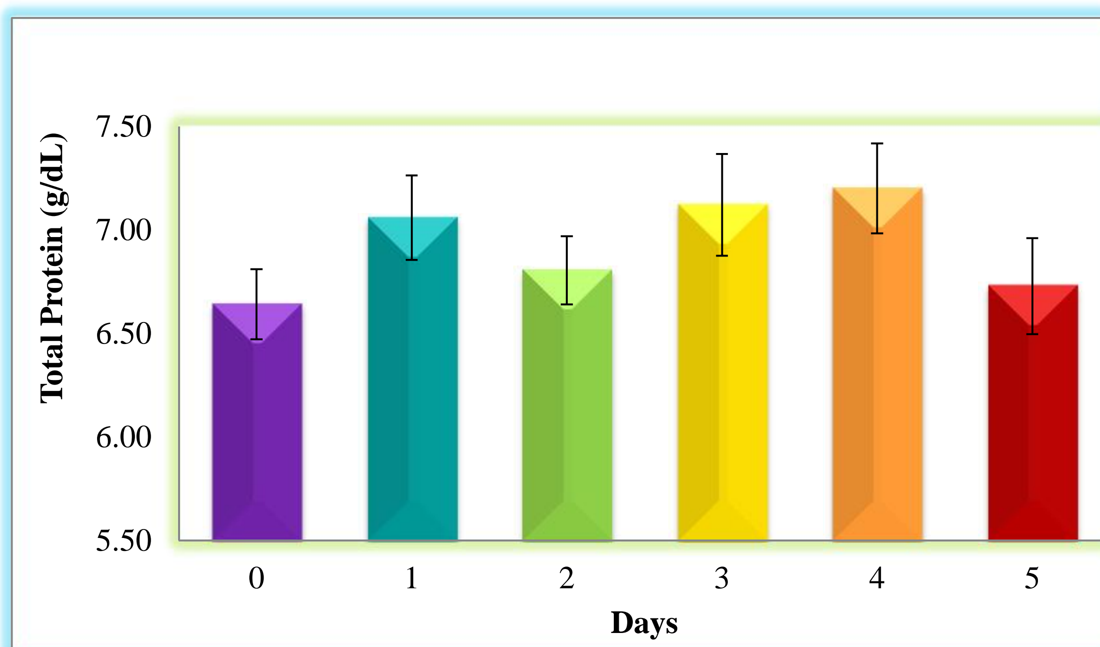

Figure 4.18: Effect of intramuscular administration of cefquinome (2 mg/kg) and tolfenamic acid (2 mg/kg) repeated at 24 h for 5 days on serum total protein level (g/dL) in sheep.

CHAPTER - V

DISCUSSION

Antimicrobials are an important class of drugs largely used to treat bacterial diseases. With the increasing awareness regarding efficacy of antibacterials and development of resistance, numerous antibacterials have been synthesized for use in different diseases of farm animals. Cephalosporins are widely used in veterinary medicine for preventing and treating bacterial infections. A major advantage of the - lactam antibiotics is their high degree of safety in the target animals. Cefquinome, an aminothiazolyl fourth generation cephalosporin, has been developed solely for veterinary use. It has certain clinical and pharmacological advantages over other cephalosporins. The advantages of cefquinome over the earlier cephalosporins include extended spectrum - lactam activity, penetration ability into the periplasmic space of Gram-negative bacteria, enhanced binding with penicillin-binding proteins and stability against -lactamases and enhanced bioavailability and potency. It is highly active against wide range of Gram-negative and Gram-positive bacteria. High safety, very low development of resistance and overall broad antibacterial activity adds to its wide usage. It has least profile of adverse reactions in animals.

Pharmacokinetic studies of cefquinome have been conducted in mice, dogs, pigs and calves (Limbert *et al*., 1991), Cows and Goats (Tohamy *et al.,* 2006), Piglets (Li *et al*., 2008), Pigs (Yang *et al*., 2009), Camels (Al-Taher, 2010), Ducks (Yuan *et al*., 2011), Sheep (Tohamy, 2011), New Forest Ponies (Smiet *et al*., 2012), Goats (Dumka *et al*., 2013), Buffalo calves (Dinakaran *et al*., 2013), Chickens (Xie *et al*., 2013) and Piglets (Song *et al*., 2013) and have reported difference between species in the disposition of cefquinome. Considering the safety and effectiveness of cefquinome, it is prudent to investigate its pharmacokinetics in target animal species and climate, in which it is to be employed clinically with a view to adopt the drug in

veterinary medicine. Under field conditions, the management of bacterial infections with the administration of antibacterial with analgesic and antipyretic agents is standard treatment. Moreover, disease condition like liver and kidney dysfunction also alter pharmacokinetic of drug disposition. Many studies proved that pharmacokinetics of an antibacterial drug may change when administered with anti-inflammatory drug (Rao *et al.,* 2000; Varma *et al*, 2000; Kumar *et al.,* 2002; Kumar *et al*., 2003; Ahmed *et al*, 2003; Waxman *et al.,* 2003; Ogino *et al.,* 2005; Sidhu *et al*. 2005; Ismail, 2006; Verma and Roy, 2006; Dumka, 2007; Dumka *et al*., 2008; Rahal *et al.,* 2008; Patel, 2009[b]) and in febrile animals (Rao *et al.,* 2000; Kumar *et al.,* 2002; Waxman *et al.,* 2003; Ismail, 2006; Verma and Roy, 2006; Patel, 2009[b]). In veterinary practice, tolfenamic acid has been used extensively in humans and in dog, cat, calf and pig medicine for its anti-inflammatory, analgesic and anti-pyretic properties. Tolfenamic acid is also used clinically as an anti-inflammatory and analgesic agent for the therapy of locomotor diseases in the dogs (Robertson and Taylor, 2004) and febrile syndromes and post-operative pain in cats (Slingsby and Waterman-Pearson, 2000). Looking to the above observations, study on effect of tolfenamic acid on pharmacokinetics of cefquinome was planned in present study in sheep.

Trikatu churna is one of the traditional poly herbal preparations, formed by mixing equal quantities of three important spicy materials such as *Piper longum*, *Piper nigrum* and *Zingiber officinale*. Piperine, obtained from the oleoresin in the peppercorns is by far the most studied and researched bioenhancer. Piperine, an alkaloid from *Piper nigrum and Piper longum* is known as a bioenhancer, which increases the bioavailability of drugs like phenytoin (Bano *et al*., 1987), propranolol and theophylline (Bano *et al*., 1991), rifampicin (Atal, 1985), oxytetracycline (Singh *et al.,*2005), pefloxacin (Dama *et al.,* 2008) and ciprofloxacin and isoniazid (Nduka *et*

al., 2013). Considering the above fact this study was planned to investigate bioenhancing effect of trikatu following intra muscular administration of cefquinome.

Despite the great potential for clinical use of cefquinome, the data on safety profile of cefquinome in sheep are not available. Looking to these overall points, the present study was planned to evaluate the effect of intramuscular administration tolfenamic acid (2 mg/kg body weight) and bioenhancers trikatu on pharmacokinetics of cefquinome (2 mg/kg body weight) following intramuscular administration in sheep; and safety of intramuscular administration of cefquinome alone (2 mg/kg body weight) and in combination with intramuscular administration of tolfenamic acid (2 mg/kg body weight) for 5 days in sheep.

5.1 Plasma levels and pharmacokinetics of cefquinome following single dose intramuscular administration in healthy sheep.

5.1.1 Plasma levels of cefquinome

For a successful antibacterial therapy, the plasma/serum concentration of an antimicrobial agent should not fall below the minimum inhibitory concentration (MIC) during the course of treatment. It is, therefore, mandatory to measure the plasma/serum drug concentrations at various time intervals after administration of an antimicrobial agent in experimental animals. The plasma drug concentrations so determined are utilized to compute various pharmacokinetic parameters, which are ultimately employed for the determination of suitable dosage regimens of a drug in a particular species for proper treatment of disease condition.

In the present study, cefquinome was administered (2 mg/kg body weight) intramuscularly in healthy sheep. Pharmacokinetic studies of cefquinome have been carried out following intramuscular administration of cefquinome at a dose rate of 1 mg $kg^{-1}$and 2 mg kg^{-1} in sheep (Tohamy, 2011; Uney *et al*., 2011), 2 mg kg^{-1} and 1 mg kg^{-1} in goats (Dumka *et al.,* 2013; Tohamy *et al*., 2006), 2 mg kg^{-1} and 1 mg kg^{-1} in buffalo calves (Dinakaran *et al*., 2013; Tohamy *et al*., 2006), 1 mg kg^{-1} in Cattle calves and cows (Tohamy *et al*., 2006), 1 mg kg^{-1} in camels (Al-Taher, 2010), 2 mg kg^{-1} in rabbit (Hwang *et al*., 2011), 5 mg kg^{-1} in ducks (Yuan *et al*., 2011), 1 mg kg^{-1} and 2 mg kg^{-1} in pigs (Yang *et al*., 2009; Lu *et al*., 2007), 2 mg kg^{-1} in piglets (Song *et al*., 2013; Li *et al,* 2008) and 2 mg kg^{-1}in chickens (Xie *et al*., 2013).

Following single dose intramuscular administration of cefquinome in sheep at the dose rate of 2 mg/kg in the present study, the peak level of 4.36 ± 0.10 μg ml^{-1} were found at 0.75 h and the concentration of cefquinome 0.1 μg ml^{-1} was maintained in plasma from 0.083 to 18 h. Similar peak plasma cefquinome

concentration of 4.57 ± 0.338 and 2.60± 0.14 µg ml^{-1} (Tohamy. 2011; Uney *et al.*, 2011) in sheep, 4.39 ± 0.53 µg ml^{-1} in piglets (Song *et al.*, 2013), 3.36 ± 0.54 µg ml^{-1} in pigs (Lu *et al.*, 2007), 4.84 ±0.23 µg ml^{-1} in goats (Dumka *et al.*, 2013) and 3.04 ± 0.71 µg ml^{-1} in chickens (Xie *et al.*, 2013) have been reported. However, lower peak plasma cefquinome concentration of 0.65 ± 0.01 µg ml^{-1} in buffalo calves (Tohamy *et* al., 2006), 0.76 ± 0.09 µg ml^{-1} in cattle calves (Tohamy *et al.*, 2006), 0.58 ±0.08 µg ml^{-1} in cows (Tohamy *et al.*, 2006) and 1.23 µg ml^{-1} in camels (Al-Taher, 2010) were reported. Hwang *et al.,* (2011) and Yuan *et al.,* (2011) reported variation in peak serum concentration of 8.87 ± 2.07 and 9.38 ± 1.61 µg ml^{-1} in rabbit and ducks respectively, following single dose intramuscular administration of cefquinome.

The maximum concentration (C_{max}) of cefquinome observed in present study is higher to that observed in buffalo calves (Tohamy *et al.*, 2006), cattle calves (Tohamy *et al.*, 2006), cows (Tohamy *et* al., 2006) and camels (Al-Taher, 2010). As the therapeutically effective concentrations is maintained in plasma up to 18 h after intramuscular administration. Thus cefquinome can be administered parentrally for the treatment of systemic infectious diseases in sheep.

5.1.2 Pharmacokinetics of cefquinome

The plasma drug concentrations of cefquinome measured at various time intervals following single dose intramuscular administration in normal sheep was employed for the calculation of various pharmacokinetic parameters like distribution half-life, elimination half-life, apparent volume of distribution, volume of distribution at steady state, total body clearance and mean residence time of the drug.

The distribution half life ($t_{1/2}$) of cefquinome following single dose intramuscular administration in the present study was 2.46 ± 0.10 h which was higher than the distribution half life of 0.31 ± 0.05 h reported in sheep (Uney *et al.*, 2011),

0.88 ± 0.42 h reported in piglets (Li *et al*., 2008) and 0.58 ± 0.27 h in chickens (Xie *et al*., 2013).

The elimination half life ($t_{1/2}$) of cefquinome following single dose intramuscular administration at the dose rate of 2 mg kg^{-1} in sheep was calculated to be 12.29 ± 2.62 h. The value is in accordance to the elimination half life of 12.86 ± 1.60 h in Buffalo Calves (Tohamy *et al.,*2006), 13.46 ± 1.80 h in cattle calves (Tohamy *et al.,* 2006), 10.24 h in camel (Al-Taher, 2010) and 8.68 ± 0.91 h in goats (Tohamy *et al.,* 2006). However, lower elimination half-life of 1.88 ± 0.40 h in sheep (Uney *et al*., 2011), 2.41 ± 0.19 h in sheep (Tohamy *et al.,* 2006), 5.86 ± 0.29 h in goats (Dumka *et al*., 2013), 1.35± 0.20 h in chickens (Xie *et al*., 2013), 1.56 ± 0.02 h in piglets (Song *et al*., 2013), 1.79 ± 0.13 h in ducks (Yuan *et al*., 2011) and 1.04 ± 0.22 h in rabbits (Hwang *et al*., 2011) have been reported. The elimination half-life of cefquinome following intramuscular administration observed in the present study indicates that the drug gets continuously absorbed during elimination phase.

To know the extent of penetration of drug in body tissue and to compute optimal dosage regimen of a drug that must be given to produce and maintain its therapeutic concentration in body, knowledge of volume of distribution is necessary. The mean apparent volume of distribution (Vd_{area}) calculated following single dose intramuscular administration of cefquinome (2 mg kg^{-1}) in the present study was 2.07 ± 0.36 L kg^{-1} which is higher than 0.51 ± 0.05 L kg^{-1} in goats (Dumka *et al*., 2013) and volume of distribution at steady state (Vd_{ss}) calculated following single dose intramuscular administration of cefquinome (2 mg kg^{-1}) in the present study was 1.07 ± 0.17 L kg^{-1} which is higher than 0.37 ± 0.02 L kg^{-1} in goats (Dumka *et al*., 2013) and 0.49 ± 0.05 L kg^{-1} in chickens. The higher values of

apparent and steady state volume of distribution following intramuscular administration of cefquinome in sheep indicate high distribution of drug in the body.

The area under serum/plasma concentration time curve (AUC) is an important parameter used to calculate clearance, volume of distribution and bioavailability of drugs in pharmacokinetic studies. The area under curve following intramuscular administration of drug in present study, the value of area under curve was 16.65 ± 0.57 µg h ml^{-1} which corroborates with the value of 13.170 ± 1.08 µg h ml^{-1} in sheep (Tohamy, 2011), 19.82 ± 2.07 µg h ml^{-1} in goats (Dumka *et al.,* 2013), 14.36 ± 1.2 µg h ml^{-1} in goats (Tohamy *et al.,* 2006), 17.22 ± 4.11 µg h ml^{-1} in pigs (Lu *et al.,* 2007), 17.76 ± 1.97 µg h ml^{-1} in buffalo calves (Tohamy *et al.,* 2006) and 18.67 ± 1.07 in cattle calves (Tohamy *et al.,* 2006). However, lower AUC values of 5.19 ± 0.25 µg h ml^{-1} in sheep (Uney *et al.,* 2011), 5.13 ± 1.06 µg h ml^{-1} in chickens (Xie *et al.* 2013) and 8.92 ± 0.53 µg h ml^{-1} in cows (Tohamy *et al.,* 2006) have been reported. Whereas higher AUC values of 23.78 ± 3.87 µg h ml^{-1} in ducks (Yuan *et al*., 2011) has been reported.

The total body clearance is an important pharmacokinetic parameter that gives sum of the clearance by each elimination organs. The total body clearance of cefquinome in present study following intramuscular administration of drug was 0.12 ± 0.01 L $h^{-1}kg^{-1}$. It is similar to the reported values 0.25 L $h^{-1}kg^{-1}$ in Pigs (Yang *et al.,* 2009).

The time required for an intact drug molecule to transit through body is termed as mean residence time (MRT). The mean residence time calculated following single dose intramuscular administration in present study was 9.14 ± 1.83 h which is in agreement with the reported values of 8.15 ± 0.98 h in sheep (Tohamy, 2011) and

8.08 ± 0.50 h in goats (Dumka *et al.,* 2013). Higher MRT values of 16.74 h in camels (Al-Taher, 2010), 21.96 ± 1.01 h, 20.8 ± 1.61 h, 11.8 ±1.07 h and 18.9 ± 1.0 h have been reported in buffalo calves, cattle calves, cows and goats respectively (Tohamy *et al*., 2006). Lower MRT of 2.05 ± 0.21 and 5.24 ± 1.12 h were reported in piglets (Song *et al.,* 2013) and sheep (Uney *et al.,*2011), respectively.

5.2 Plasma levels and pharmacokinetics of cefquinome following single dose intramuscular administration in tolfenamic acid treated sheep.

5.2.1 Plasma levels

In the present study, peak plasma drug concentration (C_{max}) of 4.73 ± 0.05 μg ml^{-1} observed in tolfenamic acid treated sheep was significantly higher than C_{max} (4.36 ± 0.10 μg ml^{-1}) observed in healthy sheep. Several author reported increase in plasma levels of different cephalosporins administration with anti inflammatory drugs. Similar significant increase in peak serum levels of cefepime following co-administration of ketoprofen has been reported (Patel *et al.,* 2012[b]). Significant increase in peak plasma levels of ceftizoxime following concomitant intramuscular administration of paracetamol with ceftizoxime has been observed in cross-bred calves (Singh *et al*., 2008). Significant increase in peak serum concentration of cefazolin at 1, 2, 4 and 6 h after intramuscular administration of phenylbutazone also reported in rabbits (Carbon *et al*., 1981). Enhanced concentrations of cefotiam and ceftriaxone following concomitant administration of diclofenac with cefotiam, cefmenoxime and ceftriaxone in rabbits have been observed (Joly *et al*., 1988).

5.2.2 Pharmacokinetics of cefquinome

Following intramuscular administration of cefquinome in tolfenamic acid-treated sheep, few of the pharmacokinetic parameters were significantly altered from that of healthy sheep. In the present study following intramuscular administration of cefquinome in tolfenamic acid-treated sheep, significant decrease in absorption half life (0.61 ± 0.10 vs. 0.26 ± 0.01 h), distribution half life (2.46 ± 0.10 vs. 1.95 ± 0.06 h) was observed while significant increase in absorption rate constant (1.27 ± 0.17 vs. 2.75 ± 0.17 h^{-1}), distribution rate constant (0.28 ± 0.01 vs. 0.36 ± 0.01 h^{-1}) and maximum drug concentration (4.36 ± 0.10 vs. 4.73 ± 0.05 µg ml^{-1}) while all other pharmacokinetic parameters were not significantly altered compared to healthy sheep. Significant increase in AUC (39.2 ± 2.09 vs.74.1 ± 2.01 µg h ml^{-1}) and $t_{1/2}$ (1.44 ± 0.12 vs. 4.08 ± 0.54 h) has been reported following concomitant administration of paracetamol with ceftizoxime in crossbred calves (Singh *et al.*, 2008). Significant increase in absorption half life (0.16 ± 0.01 vs. 0.22 ± 0.0 h) of cefepime was reported following co-administration with ketoprofen in sheep (Patel *et al.*, 2012[b]). Significant increase in elimination half life (2.29 ± 0.08 vs 2.09 ± 0.08) was observed following concomitant administration of cefoperazone with ketoprofen in goats (Barot *et al.*, 2011). Significant increase in the value of elimination half life ($t_{1/2}$) (0.87 ± 0.016 to 1.42 ± 0.092 h) of cefazolin was reported following co-administration with phenylbutazone in rabbits (Carbon *et al.,* 1981). Significant increase in AUC of ceftriaxone (326.0 ± 91.4 vs. 555.0 ± 124.0 µg h ml^{-1}), cefotiam (17.5 ± 4.4 vs. 32.9 ± 17.9 µg h ml^{-1}) and half life of ceftriaxone (2.8 ± 0.5 vs. 3.45 ± 0.4 h) and significant decrease in clearance of cefotiam (23.7 ± 6 to 17 ± 5 ml min^{-1}) has been reported in rabbits (Joly *et al.,* 1988).

5.3 Plasma levels and pharmacokinetics of cefquinome following single dose intramuscular administration in trikatu pre-treated sheep

The present study was undertaken to evaluate bioenhancing effect of pulverized powder of trikatu on pharmacokinetics of cefquinome in sheep.

5.3.1 Plasma levels of cefquinome

Following intramuscular administration (2 mg/kg body weight) of cefquinome in trikatu pretreated sheep, peak plasma drug concentration (C_{max}) of 5.23 ± 0.08 µg/mL at 0.75 h (T_{max}) were significantly higher than the healthy sheep C_{max}: (4.36 ± 0.10 µg/mL).

Principle content of trikatu is Piperine (1-piperoyl piperidine), an amide alkaloid, from a different species of pepper which are ingredient (*Piper longum* and *Piper nigrum*) of trikatu, and was mainly responsible for enhancing the plasma concentration of concurrently administered drugs (Atal, 1979; Mathur *et al*., 1998). Plasma drug concentrations in elimination phase (8 h) were significantly higher following intramuscular cefquinome administration in trikatu pretreated sheep than healthy sheep. Similar results were observed in gaddi goats following oral administration of pefloxacin in trikatu pretreated goats (Dama *et al*., 2008). The results obtained in present study were also in accordance with higher plasma concentrations reported following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep (Walunj, 2008). Significant higher plasma concentrations following oral administration of levofloxacin (4 mg/kg) in trikatu preteated goats was also reported (Patel, 2012). Higher plasma concentrations following oral administration of oxytetracycline along with *Piper longum* in hens were also observed (Singh *et al*., 2005). In accordance to present study trikatu ME, *Piper nigrum* ME and *Piper longum* ME have shown elevated plasma level of ampicillin following oral

dosing in rabbits (Janakiraman and Manavalan, (2008[b]). Janakiraman and Manavalan, (2008[a]) have found significantly increased level of peak plasma concentration of norfloxacin in piperine treated group (17.78 ± 0.32 µg/ml) than norfloxacin alone group (7.07 ± 0.26 µg/ml) at 2 hours in rabbits. Several studies have reported enhancement of plasma levels of drugs like vasicine, sparteine, phenytoin, propranolol, theophylline, rifampicin, sulphadiazine and tetracycline when coadministered with trikatu or piperine (Zutshi *et al*., 1985; Bano *et al*., 1987; Bano *et al*., 1991).

In contrast to present study reduction in plasma concentration of isoniazid and rifampicin in rabbits were observed (Karan *et al*., 1998; Karan *et al*., 1999[b]). The above observed variation in results may be due to differences in species and class of drug.

5.3.2 Pharmacokinetics of cefquinome

The plasma drug concentrations measured at various time intervals in trikatu pretreated sheep following single dose intramuscular administrations of cefquinome at the rate of 2 mg/kg body weight were employed for the calculation of various pharmacokinetic parameters of the drug.

Following single dose intramuscular administration of cefquinome in trikatu pretreated sheep, the absorption half-life ($t_{1/2ka}$), was found to be 0.45 ± 0.04 h in healthy group. Similarly, Dama *et al*., (2008) have observed no alteration in absorption half life following pefloxacin administration in trikatu pretreated goats. Similarly, Singh *et al*., (2005) observed no alteration in absorption half-life ($t_{1/2ka}$) following oxytetracycline dosing in hens along with *Piper longum*. Similarly, Patel (2012) reported no significant alteration in absorption half-life ($t_{1/2ka}$) following oral administration of levofloxacin (4 mg/kg) in trikatu pretreated goats. Walunj, 2008

also reported no significant alteration in absorption half-life ($t_{1/2ka}$) following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep.

The elimination rate constant (β) of cefquinome following single dose intramuscular administration (2 mg/kg body weight) in trikatu pretreated sheep was 0.09 ± 0.01 h^{-1}. Similarly, Walunj (2008) reported no significant alteration in the elimination rate constant. However, decrease in elimination rate constant (β) has also been reported (Patel, 2012; Dama *et al*., 2008).

The maximum drug concentration (C_{max}) of cefquinome following single dose intramuscular administration (2 mg/kg body weight) in trikatu pretreated sheep (5.23 ± 0.08 μg ml^{-1}) was significantly ($P<0.05$) higher than healthy sheep (4.36 ± 0.10 μg ml^{-1}). The results were also in accordance with results as reported following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep (Walunj, 2008). Similarly, in trikatu pretreated goats significantly higher (C_{max}) was observed following oral administration (4 mg/kg) of levofloxacin (Patel, 2012).

The value of area under plasma concentration time curve (AUC) following single dose intramuscular administration of cefquinome (2 mg/kg body weight) in trikatu pretreated sheep was (18.89 ± 0.06 μg·h/mL) highly significant than healthy sheep (16.65 ± 0.57 μg·h/mL). Similarly, significantly higher value of AUC was observed following pefloxacin administration in trikatu pretreated goats. Moreover, Janakiraman and Manavalan, (2008[a]) and Bhise and Pore, (2002) also reported higher value of area under curve following oral administration of norfloxacin and ciprofloxacin along with piperine in rabbits. In addition to these Singh *et al*. (2005) observed higher AUC in *Piper longum* pretreated hens following oxytetracycline administration. High AUC values in present study may be due to inhibition of

enzymes responsible for metabolism of drug in liver and inhibition of renal secretion or elimination, which leads to more free drug concentration for longer duration in body (Atal *et al.*, 1985; Sweetman, 2002). The results were also in agreement with increased AUC as reported following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep (Walunj, 2008). Similarly, significantly high rise in AUC was observed in trikatu pretreated goats following oral administration (4 mg/kg) of levofloxacin (Patel, 2012).

The apparent volume of distribution (Vd_{area}) of cefquinome following single dose intramuscular administration (2 mg/kg body weight) in trikatu pretreated sheep (1.27 ± 0.12 L/kg) was not significantly altered. Similary, no significant alteration was observed following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep (Walunj, 2008). Whereas, increased Vd_{area} have also been recorded in trikatu pretreated goats (Dama *et al*., 2008; Patel, 2012).

Following single dose intramuscular administration (2 mg/kg body weight) in trikatu pretreated sheep, the elimination half-life ($t_{1/2\beta}$) was found to be 8.33 ± 0.79 h. Similarly, no significant alteration was observed following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep (Walunj, 2008). Whereas, increased elimination half-life was observed in trikatu pretreated goats following oral administration (4 mg/kg) of levofloxacin (Patel, 2012). These studies indicated that piperine caused concentration related inhibition of NADPH- dependent cytochrome P-450 oxidase enzymes which play a central role in disposition, steady state balance of drugs and xenobiotics. In addition, piperine is a strong inhibitor of UDP-glucoronyl transferase enzyme and impaired down regulation of Cytochrome P-450 1A1 gene expression in the rat heaptoma 5 L cell lines. It was due to direct interaction of the

alkaloid with Cytochrome P-4501A1 enzyme at post – translation level (Guido *et al*., 1998).

Mean residence time is average total time during which molecules of a given dose remain in the body. In present study, no significant alteration in mean residence time was recorded following intramuscular administration of cefquinome (2 mg/kg body weight) in trikatu pretreated sheep than healthy sheep (9.14 ± 1.83 vs. 7.06 ± 0.39 h). The result was in accordance with results as reported following oral administration of gatifloxacin (10 mg/kg) in trikatu pretreated sheep (Walunj, 2008). However, higher MRT have been reported in trikatu pretreated goats (Dama *et al*., 2008; Patel, 2012).

5.4 Safety assessment of multiple intramuscular administrations of cefquinome in combination with tolfenamic acid in sheep.

Antimicrobial therapy is usually employed to cure bacterial infection or to prevent the secondary bacterial infection. Prolonged therapy or high dosage regimen implemented during the treatment may exhibit the toxic effect, if any. Considering this fact, safety study of a drug is indeed necessary to notify any alteration in the physiological or biochemical parameters of the body fluid and tissue along with therapeutic effects. If alterations are very minor, the drug is relatively safe and if severe, then adverse effects of such drug will observed. The effects of long term cefepime therapy on body systems were extensively evaluated in rats and dogs (Kadota *et al.,* 1990; Kadota *et al.,* 1992).

Safety of multiple intramuscular doses of cefquinome (2 mg/kg body weight) in combination with intramuscular administration of tolfenamic acid (2 mg/kg body weight) repeated at 24 hours interval in healthy sheep was monitored by studying various hematological and blood biochemical evaluations. The hematology included

determination of hemoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC). The results suggest that multiple intramuscular doses of cefquinome (2 mg/kg body weight) in combination with intramuscular administration of tolfenamic acid (2 mg/kg body weight) repeated at 24 hours interval in healthy sheep did not alter the hematological parameters. It indicates that repeated long term administration of cefquinome in combination with tolfenamic acid was well tolerated in sheep. Similarly, Patel *et al.* (2012[b]) reported no significant changes in haematological parameters following repeated intravenous cefepime administration (20 mg kg^{-1}) in sheep. Barot *et al* (2011) reported no significant changes in haematological and biochemical parameter cefpirome in combination with intramuscular administration of ketoprofen. Kadota *et al.* (1992) reported slight decrease in the average value of relative lymphocyte counts and a slight increase in the average value of relative segmented neutrophil counts.

Various serum enzymes like asparate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AKP), lactate dehydrogenase (LDH) and bilirubin (total) were determined during the course of treatments to evaluate patency of liver functions following multiple-dose intramuscular administration of cefepime in combination with ketoprofen in sheep. Similarly serum creatinine and blood urea nitrogen levels were determined to monitor the patency of renal function.

In the present study, no significant alterations ($p<0.05$) in all blood biochemical parameters have been observed following daily intramuscular administration of cefquinome in combination with tolfenamic acid repeated at 24 hr interval for 7 days in sheep. Patel *et al.*, (2012[a]) found no significant alterations in blood biochemical parameters following daily intramuscular administration of

cefepime in combination with ketoprofen repeated at 12 hr interval for 5 days in goat. Patel *et al*., (2012[b]) have found no significant alteration in all blood biochemical parameters except ALT and AST values measured at 108 and 120 h following repeated intravenous cefepime administration (20 mg kg^{-1}) in sheep. High dose cefoperazone administration has demonstrated hepatic and renal toxicity in laboratory animals. The average organ weight including the liver, brain, lungs were decreased following cefoperazone therapy at the rate of 1500 mg kg^{-1} for 28 days in rats (Kadota *et al.,* 1990). Reversible hepatomegaly (Donowitz, 1989) and hepatic damage with elevated ALT and AST have been noted during the cephalosporin therapy at higher dose rate (Kalman and Barriere, 1990). Higher dose of cephalothin (>12 g day^{-1}, total dose) was found to cause nephrotoxicity in rabbits (Rankin and Sutherland, 1989). Kai *et al.* (1992) has noticed increase in kidney weights in F1 generation with no alteration in creatinine level in rats treated with cefepime at dose rate of 1000 mg kg^{-1} daily for 63 days.

On the basis of observation of various haematological and blood biochemical parameters, multiple intramuscular administration of cefquinome along with tolfenamic acid did not affect hepatic as well as renal functioning. This suggests that long term intramuscular administration of cefquinome along with tolfenamic acid is safe in sheep. It is advisable to use cefquinome along with tolfenamic acid at recommended dosage for treatment of infections not responding to conventional antibacterial drugs.

CHAPTER - VI

SUMMARY & CONCLUSION

The present study was planned to determine the effect of intramuscularly administered tolfenamic acid (2 mg kg^{-1} of body weight) on pharmacokinetics of cefquinome following intramuscular administration (2 mg kg^{-1} of body weight) in sheep. Moreover, effect of bioenhancer trikatu on pharmacokinetics of cefquinome following intramuscular administration (2 mg kg^{-1} of body weight) in sheep was evaluated. Finally, safety of daily intramuscular administration of cefquinome (2 mg kg^{-1} of body weight at 12 h interval) in combination with intramuscular administration of tolfenamic acid (2 mg kg^{-1} of body weight at 24 h interval) for five days in sheep was also carried out by monitoring haematological and blood biochemical profiles. Drug concentration in plasma was determined by using High Performance Liquid Chromatography (HPLC).

Following single dose intramuscular administration of cefquinome at the rate of 2 mg kg^{-1} of body weight in sheep, the drug concentration (0.49 ± 0.01 μg ml^{-1}) was detectable at 0.083 hours (5 minutes) and peak plasma concentration (4.36 ± 0.10 μg ml^{-1}) attained at 0.75 h. The plasma drug concentration ≥ 0.13 μg/ml was detected up to 18 h. Very short absorption half-life ($t_{1/2ka}$) of 0.61 ± 0.10 h and long elimination half-life ($t_{1/2\beta}$) of 12.29 ± 2.62 h observed in the present study indicated that the drug is rapidly absorbed and slowly eliminated after intramuscular administration in sheep. Based on the plasma cefquinome concentrations, various pharmacokinetic parameters like apparent volume of distribution (Vd_{area}) (2.07 ± 0.36 L kg^{-1}), total body clearance (Cl_B) (0.12 ± 0.01L h^{-1} kg^{-1}), area under plasma drug concentration-time curve (AUC) (16.65 ± 0.57 μg h ml^{-1}), area under first moment of

curve (AUMC) (157.05 ± 37.93 µg h^2 ml^{-1}) and mean residence time (MRT) (9.14 ± 1.83 h) were determined.

Following single dose intramuscular administration of cefquinome in tolfenamic acid-treated sheep, the drug concentration (0.51 ± 0.01 µg ml^{-1}) was detectable at 0.083 hours (5 minutes) and peak plasma concentration (4.73 ± 0.05 µg ml^{-1}) attained at 0.75 h. The plasma drug concentration ≥ 0.15 µg/ml was detected up to 18 h. The absorption half-life ($t_{1/2ka}$), elimination half-life ($t_{1/2\beta}$), apparent volume of distribution (Vd_{area}), total body clearance (Cl_B) and mean residence time (MRT) were 0.26 ± 0.01h, 9.00 ± 0.51 h, 1.48 ± 0.08 L kg^{-1}, 0.11 ± 0.01 L h^{-1} kg^{-1} and 7.27 ± 0.27 h, respectively. The average values for area under plasma drug concentration-time curve ($AUC_{0-\infty}$) and area under first moment curve (AUMC) were 17.52 ± 0.14 µg h ml^{-1} and 127.55 ± 5.24 µg h^2 ml^{-1}, respectively. Significant alteration in absorption rate constant, distribution rate constant, absorption half-life, distribution half life and peak plasma drug concentration of tolfenamic acid-treated sheep has been observed compared to healthy sheep while other pharmacokinetics parameters were not significantly altered in tolfenamic acid-treated sheep compared to healthy sheep.

In trikatu pretreated sheep drug was detected upto 18 hours. Plasma drug concentrations were highly significantly from 0.166 to 18 h post cefquinome administration in trikatu pretreated sheep than healthy sheep. The plasma drug concentration (0.52 ± 0.01µg ml^{-1}) was detectable at 0.083 hours (5 minutes) and peak plasma concentration (5.23 ± 0.08 µg ml^{-1}) attained at 0.75 h. The plasma drug concentration ≥ 0.16 µg/ml was detected up to 18 h. Following intramuscular administration of cefquinome in trikatu pretreated sheep, the absorption half-life

($t_{1/2ka}$), elimination half-life ($t_{1/2\beta}$), apparent volume of distribution (Vd_{area}), total body clearance (Cl_B) and mean residence time (MRT) were 0.45 ± 0.04 h, 8.33 ± 0.79 h, 1.27 ± 0.12 L kg^{-1}, 0.11 ± 0.01 L h^{-1} kg^{-1} and 7.06 ± 0.39 h, respectively. Significant alteration in distribution half-life ($t_{1/2\alpha}$), total body clearance ($Cl_{(B)}$), maximum drug concentration (C_{max}) and area under curve ($AUC_{(0-)}$) of trikatu pretreated sheep has been observed compared to healthy sheep while other pharmacokinetics parameters were not significantly altered in trikatu pretreated sheep compared to healthy sheep.

Following daily intramuscular administration of cefquinome (2 mg kg^{-1}) in combination with intramuscular administration of tolfenamic acid (2 mg kg^{-1}) for 5 days, no significant alterations ($p<0.05$) in all haematological and serum biochemical parameters were observed in sheep. All the parameters were found to fluctuate within normal range during treatment and the values were not significantly different from corresponding control values (0 day). It suggests that repeated administration of cefquinome (2 mg kg^{-1}) in combination with intramuscular administration of tolfenamic acid (2 mg kg^{-1}) in sheep is safe as no clinical symptoms of adverse reaction or toxicity was observed.

Following conclusions can be drawn from the present study:

1) The plasma drug concentrations of cefquinome following its intramuscular administration in tolfenamic acid-treated sheep were significantly higher during the phase of elimination compared to respective levels in healthy sheep.
2) Co-administration of tolfenamic acid significantly increased peak plasma level (C_{max}) of cefquinome in sheep.
3) Following intramuscular administration of the drug in healthy sheep, the mean elimination half-life ($t_{½}$), apparent volume of distribution (Vd_{area}) and total body clearance (Cl_B) were 12.29 ± 2.62 h, 2.07 ± 0.36 L kg^{-1} and 0.12 ± 0.01 L h^{-1} kg^{-1}, respectively.
4) After intramuscular administration of the drug, significant increases in absorption rate constant (K_a) and distribution rate constant were observed in tolfenamic acid-treated compared to healthy sheep.
5) The plasma drug concentrations of cefquinome following its intramuscular administration in trikatu pretreated sheep were highly significant during the phase of elimination compared to respective levels in healthy sheep.
6) After intramuscular administration of the drug, significant increase in AUC and C_{max} of the drug observed in trikatu pretreated sheep compared to healthy sheep.
7) Cefquinome (2 mg/kg, IM) in combination with tolfenamic acid (2 mg/kg, IM) was found safe following intramuscular administration repeated at 24 hour interval for consequent 5 days based on evaluation of the hematological and serum biochemical parameters.

REFERENCES

Abd El-Aty, A.M. and Goudah, A. (2002). Some pharmacokinetic parameters of pefloxacin in lactating goats. *Vet. Res. Commun.* **26**: 553-561.

Ahmed, A.; Gaber, A.; Farghaly, O. A.; Ghandour, M. A. and El-Said, H. S. (2000). Potentiometric Studies on Some Cephalosporin Complexes. Monatshefte fur Chemie/Chemical Monthly. **131(10):** 1031-1038.

Ahmed, F.A.; Mohan, P.; Barua, C.C. and Dutta, D.J. (2003). Effect of intramuscular diclofenac sodium on pharmacokinetics of intravenous enrofloxacin in calves. *Indian J. Pharmacol.* **37**: 189-190.

Al-Rawithi, S.; Hussein, R.; Raines, D. A.; Al-Showaier, I. and Kurdi, W. (2000). Sensitive assay for the determination of cefazolin or ceftriaxone in plasma utilizing LC. *J. Pharmaceut. Biomed. Anal*. **22(2):** 281-286.

Al-Taher, A. Y. (2010). Pharmacokinetics of Cefquinome in Camels. *J. Anim. Vet. Adv.* **9 (4)**: 848-852

Amirids, G. S.; Fthenakis, G. C.; Dafopoulos, J.; Papanikolaou, T. and Mavrogianni, V. S. (2003). Use of cefquinome for prevention and treatment of bovine endometritis. *J. Vet. Pharmacol. Ther.* **26:**387–390.

Annamalai, A.R. and Manavalan, R. (1989). Effects of Trikatu and its individual components and piperine on gastrointestinal tract: Trikatu - A bioavailability enhancer. *Indian Drugs.* **27**:595-604.

Annamalai, R and Manavalan, R. (1990). Effects of ‘Trikatu’ and its individual components and piperine on gastrointestinal tracts: Trikatu—a bioavailable enhancer. *Indian Drugs*. **27**: 595–604.

Arayne, M. S.; Sultana, N. and Nawaz, M. (2006). A RP-HPLC method for the assay of cefpirome and its applications in drug-metal interaction studies. *Pak. J. Pharm. Sci.* **19(1):**38-43.

Arayne, M. S.; Sultana, N. and Rafiq, K. (2001). In vitro activity of ceftizoxime and ceftazidime in presence of essential and trace elements. *Pak. J. Pharm. Sci.* **14(2):** 57-64.

Atal, C. K. (1979). A breakthrough in drug bioavailability - a clue from age old wisdom of Ayurveda. *Indian Drug Manuf. Asso. Bull.* 10: 483-484.

Atal, C. K.; Dubey, R. K. and Singh, J. (1985). Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. *J. Pharmacol. Exp. Ther.* **232(1):** 258-262.

Atal, C. K.; Zutshi, U. and Rao, P. G. (1981). Scientific evidence on the role of Ayurvedic herbals on bioavailability of drug. *J. Ethanopharmacol.* **4:** 117.

Atal, N. and Bedi, K. L. (2010). Bioenhancers: Revolutionary concept to market. *J Ayur Integ Med.***1**:96–99.

Baggot, J. D. (1977). Principles of drug disposition in domestic animals. The basis of veterinary Clinical Pharmacology. I[st] Edn.; W.B. Saunders Co.; Philadelphia, U.S.A, pp:144-189.

Bajad, S.; Coumar, M.; Khajuria, R.; Suri, O. P. and Bedi, K. L. (2003). Characterization of a new rat urinary metabolite of piperine by LC/NMR/MS studies. *Eur. J. Pharm. Sci.* **19**:413–421.

Bano, G.; Amla, V. and Raina R. K. (1987). The effect of piperine on kinetics of phenytoin in healthy volunteers. *Planta Medica* **53**:568-569.

Bano, G.; Raina, R. K.; Zutshi, U. and Bedi, K. L. (1991). Effect of piperine on the bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *Eur J Clin Pharmaco* **41**:615-617.

Barckow, D. and Schwigon, C. D. (1993). Cefepime versus cefotaxime in the treatment of lower respiratory tract infections. *J. Antimicrob. Chemother.* **32:** 187-193.

Barkema, H. M.; Schukken, Y. H.; Lam, T. G. M.; Beiboer, M. L.; Wilmine, H.; Benediktus, G. and Brand, A. (1998). Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *J. Dairy Sci.* **81**: 411-419.

Barot, (2011). Studies on pharmacokinetics of cefpirome in rats and effect of fever and co-administration of ketoprofen on pharmacokinetics of cefpirome along with its safety in goats. Ph.D. Thesis. Anand Agricultural University. Gujarat, India.

Barradel, L. B. and Bryson, H. M. (1994). Cefepime: A review of this antibacterial activity, pharmacokinetics properties and therapeutic use. *Drugs.* **47(3):** 471-505.

Barry, A.L.; Brown, S.D. and Novic, W.J. (1995). *In vitro* activities of cefotaxime, ceftriaxone, ceftazidune, cefpirome and penicdhn against Streptococcus pneumoniae isolates. *Antimicrob Agents Chemother.* **39:** 2193-2196.

Becker, M.; Zittlau, E. and Petz, M. (2004). Residue analysis of 15 penicillins and cephalosporins in bovine muscle, kidney and milk by liquid chromatography-tandem mass spectrometry. *Analytica Chemica Acta.***520**:19-32

Bhardwaj, R. K.; Glaeser, H.; Becquemont, L.; Klotz. U.; Gupta, S. K. and Fromm, M. F. (2002), Piperine, a major constituent of black pepper inhibits human p-glycoprotein and CYP3A4. *J. Pharmacol. Exp. Ther.* **302**: 645-650.

Bhat, B. G. and Chandrasekhara, N. (1985). Determination of piperine in biological tissues by thin-layer chromatography and ultraviolet absorption densitometry. *J. Chromatography*. **338**(1): 259-263.

Bhat, B. G. and Chandrasekhara, N. (1986). Studies on the metabolism of piperine: absorption, tissue distribution and excretion of urinary conjugates in rats. *Toxicology*. **40**(1): 83-92.

Bhat, B. G. and Chandrasekhara, N. (1987). Metabolic disposition of piperine in the rat. *Toxicology*. **44**(1): 99-106.

Bhise, S. B. and Pore, V. Y. (2002). Influence of co-administration of piperine on pharmacokinetic profile of ciprofloxacin. *Indian drugs*. **39**(3): 166-168.

Bottner, A.; Schmid, P. and Humke, R. (1995). In vitro efficacy of cefquinome (INN) and other anti-infective drugs against bovine bacterial isolates from Belgium, France, Germany, The Netherlands, and the United Kingdom. *Zentralbl. Veterinarmed.* **42**(6):377-83.

Bowman, D. B.; Aravind, M. K.; Miceli, J. N. and Kauffman, R. E. (1984). Reversed phase high performance liquid chromatographic method to determine ceftriaxone in biological fluids. *J. Chromatography*. **309(1):** 209-213.

Bryskier, A. (1997). New concepts in the field of cephalosporins: C-3' quarternary ammonium cephems (Group IV). *Clin. Microbiol. Infect.* **3** (Suppl 1):S1-S6.

Caprile, K. A. (1988). The cephalosporin antimicrobial agents: a comprehensive review. *J. Vet. Pharmacol. Ther.* **11**(1): 1-32.

Carbon, C.; Contrepois, A.; Nivoche, Y.; Grandjean, M.; Decourt, S. and Chau, N. P. (1981). Effects of phenylbutazone on extravascular diffusion, protein binding and urinary excretion of cefazolin in rabbits. *J. Pharmacol. & Exper. Therap.* **218(2):** 537-543.

Chaudhary, A. (2003). In vitro activity of Cefepime: A new fourth generation cephalosporin. *Indian J. Med. Microbiol.* **21**(1): 52-55.

Chin, N. X.; Gu, J. W.; Fang, W. and Neu, H. C. (1992). In vitro activity of cefquinome, a new cephalosporin, compared with other cephalosporin antibiotics. *Diagn. Microbiol. Infect. Dis.* **15**(4):331-7.

Curtis, N. A.; Orr, D.; Ross, G. and Boulton, M. G. (1979). Affinities of penicillins and cephalosporins for the penicillin- binding proteins of *Escherichia coli* KI2 and their antibacterial activity. *Antimicrob.Agents Chemother.,* **16**: 533-539.

Dalvi, R. R. and Dalvi, P. S. (1991). Differences in the effects of piperine and piperonyl butoxide on hepatic drug-metabolizing enzyme system in rats. *Drug Chem.Toxicol.* **14**(1-2):219-229.

Dama, M. S.; Varshneya, C.; Dardi, M. S. and Katoch, V. C. (2008). Effect of trikatu pretreatment on the pharmacokinetics of pefloxacin administered orally in mountain Gaddi goats *J. Vet. Sci.* **9**(1): 25-29.

Dinakaran, V. and Dumka, V. K. (2012). Pharmacokinetics of tolfenamic acid following two oral dose levels in buffalo calves. *J. Vet. Pharmacol Ther.* doi: 10.1111/jvp.12025.

Dinakaran, V.; Dumka, V.; Ranjan, B.; Balaje, R. and Sidhu, P. K. (2013). Pharmacokinetics following intravenous administration and pharmacodynamics of cefquinome in buffalo calves. *Trop. Anim. Health Prod.* **45(7):**1509-12.

Domenico, P.; Leary, R. O. and Cunha, B. A. (1992). Differential effects of bismuth and salicylate salts on the antibiotic susceptibility of *Pseudomonas aeruginosa*. *Eur. J. Clin. Micro. & Infec. Dis.* **11(2):** 170-175.

Donowitz, G. R. (1989). Third generation cephalosporin. *Infect. Dis. Clin. North. Am.* **3(3):** 595-612.

Dumka, V. K.; Dinakaran, V.; Ranjan, B. and Rampal. S. (2013). Comparative pharmacokinetics of cefquinome following intravenous and intramuscular administration in goats. *Small Ruminant Res.* **113(1):**273-277

Dumka, V.K. (2007). Disposition kinetics and dosage regimen of levofloxacin on concomitant administration with paracetamol in crossbred calves. *J. Vet. Sci.* **8:** 357-360.

Dumka, V.K.; Harpreet Singh and Srivastava, A.K. (2008). Disposition kinetics and urinary excretion of levofloxacin on concomitant administration with meloxicam in cross-bred calves. *Envirol. Toxicol. Pharmacol.* **26**: 56–60.

Ehinger, A. M.; Schmidt, H. and Kietzmann, M. (2006). Tissue distribution of cefquinome after intramammary and "systemic" administration in the isolated perfused bovine udder. *Vet. J.* **172**(1):147-53.

Finar, I. L. (1975). Stereochemistry and the chemistry of natural products. In: Organic Chemistry, 5th Edn., ELBS, Longman's UK publication, London, UK.

Francioli, P.; Shah, P. M.; Torres, A.; Chastre, J.; Langer, M. and Santos, J. I. (1997). Nosocomial pneumonia in the ICU: When pathogenesis helps define prevention and treatment. *Clinical Microbiology and Infection*. **3(1):** 87-101.

Fremaux, A.; Sissia, G. and Geslin, P. (1994). In vitro antibacterial activity of cefotaxime, cefpirome and four other beta-lactam antibiotics against penicillin-resistant Streptococcus pneurnoniue. 6th lilt Congr Infect Dis Prague. PCS 26.

Frere, J. M.; Joris, B.; Granier, B.; Matagne, A.; Jacob, F. and Bourguignon-Bellefroid, C. (1991). Diversity of the mechanisms of resistance to β-lactam antibiotics. *Res. Microbiol.* **142**: 705-710.

Funaki, H.; Yuji, U.; Shigeyuki, T.; Katsuaki, I.; Yasuhiro, T.; Kenichi, K. and Takao. S. (2001). Therapeutic Efficacy of Cefquinome against Acute Respiratory Disease in Holstein Steers. *Journal of the Japan Veterinary Medical Association.* **54(6)**:451-454.

Fung-Tomc, J.; Dougherty, T.J.; DeOrio, F.J.; Simich-Jacobson, V. and Kessler, R.E. (1989). Activity of cefepime against ceftazidime and cefotaxime-resistant gram-negative bacteria and its relationship to -lactamase levels. *Antimicrob. Agents Chemother.* **33(4):** 489-502.

Garau, J.; Wilson, W. W.; Wood, M. and Carlet, J. (1997). Fourth-generation cephalosporins: a review of in vitro activity, pharmacokinetics, pharmacodynamics and clinical utility. *Clinical Microbiology and Infection*. **3**(1): 87-101.

Giamarellou, H. (1996). Clinical experience with the fourth generation cephalosporins. *J. Chemother.* **8(2):** 91-104.

Goldberg, D. M. (1987). The cephalosporins (review). *Med. Clin. North. Am.* **71(6):** 1113-1133.

Graham, J.M.; Oshiro, B.T. and Blanco, J.D. (1992). Limited spectrum (first generation) cephalosporins. *Obster. Gynecol. Clin. North. Am.* **19(3):** 449-459.

Guerin-Faublee, V.; Carret, G. and Houffschmitt, P. (2003). In vitro activity of 10 antimicrobial agents against bacteria isolated from cows with clinical mastitis. *Vet. Rec.* **152**:466–471.

Guido, S.; Joy, D.; Joseph, D.; Majeed, M.; Rajendran, R. and Shrinivas, P. S. (1998). Pharmacokinetics of the curcumin in animals and human volunteers. *Planta Medica*. **64**: 353- 356.

Hancock, R. E. and Bellido, F. (1996). Factors involved in the enhanced efficacy against gram negative bacteria of fourth generation cephalosporin. *Antimicrob. Agents. Chemother.* **29**(A):1-6.

Hancock, R. E. W. and Bellido, F. (1992). Factors involved in the enhanced efficacy against gram-negative bacteria of fourth-generation cephalosporins. *J. Antimicrob. Chemother*. **29**: 1-6.

Heinritzi, K. and Hagn, J. (1999). Comparison of therapeutic Performance of the new cephalosporin cefquinome with other treatment regimes in gilts with puerperal septicemia and toxemia syndrome. *Tierarztl. Prax. Ausg. G. Grosstiere. Nutztiere.* **27**:114–121.

Heinritzi, K. and Hagn, J. (1999). Effectiveness and disposition of the newly developed cephalosporin cefquinome in puerperal septicemia and toxemia in gilts. *Tierarztl. Prax. Ausg. G. Grosstiere. Nutztiere.* **27(2)**:114-121.

Henry, R. J.; Cannon, D. C. and Winkelman, J. W. (1974). Clinical Chemistry: Principles and Techniques, 2nd Edn. Harper & Row.

Hiraoka, N.; Masuyoshi, S.; Mitsuhashi, S.; Tomatsu, K. and Inoue, M. (1988). Cephalosporinase interactions and antimicrobial activity of BMY-28142, ceftazidime and cefotaxime. *J. Antibiot.* **41(1):** 86-93.

Hiwale, A. R.; Dhuley, J. N. and Naik, S. R. (2002). Effect of co-administration of piperine on pharmacokinetics of -lactam antibiotics in rats. *Indian J. Exp. Biol.* **40:** 277-81.

Hornish, R. E. and Kotarski, S. F. (2002). Cephalosporins in veterinary medicine- ceftiofur use in food animals. *Curr. Top. Med. Chem.* **2(7):** 717-731.

Hwang, Y. H.; Song, I. B.; Lee, H. K.; Kim, T. W.; Kim, M. S.; Lim, J. H.; Park, B. K. and Yun, H. I. (2011). Pharmacokinetics and bioavailability of cefquinome in rabbits following intravenous and intramuscular administration. *J. Vet. Pharmacol. Ther.* **34**:618–620.

Ismail, M. (2006). A pharmacokinetic study of danofloxacin in febrile goats following repeated administration of endotoxin. *J. Vet. Pharmacol. Ther..* **29**: 313–316.

Itamochi, H.; Yoshida, T.; Walker, C. ; Bartholomeusz, C.; Aoki, D.; Ishihara, H.; Suzuki, N.; Junzo, K. Terakawa, N.; Ueno,N. (2011). Novel mechanism of reduced proliferation in ovarian clear cell carcinoma cells: Cytoplasmic sequestration of CDK2. *Gynecologic oncology.* p 27.

Jacoby, G. A. and Sutton, L. (1985). Beta-Lactamases and Beta-lactam resistance in Escherichia coli. *Antimicrob Agents Chemother.* **28(5):** 703–705.

Janakiraman K. and Manavalan R. (2008[a]). Studies on effect of piperine on oral bioavailability of ampicillin and norfloxacin. *Afr. J. Trad. CAM.* **5**(3): 257 – 262.

Janakiraman, K and Manavalan, R. (2008[b]). Studies on effect of co-administration of Trikatu and its components on oral bioavailability of ampicillin and norfloxacin, in rabbits. *J. Pharmacy Res.* **2**(1):27-30.

Jauregui, L.; Matzke, D.; Scott, M.; Minns, P. and Hageage, G. (1993). Cefepime as treatment for osteomyelitis and other severe bacterial infectious. *J. Antimicrob. Chemother.* **32(B):** 141-149.

Jaussaud, P.; Guieu, D.; Bellon, C.; Barbier, B.; Lhopital, M.C.; Sechet, R.; Courtot, D. and Toutain, P.L. (1991). Pharmacokinetics of tolfenamic acid in the horse. *Equine Veterinary Journal.* **23**:69–72.

Jendrassik, L. and Grof, P. (1938). Simplified photometric methods for the determination of blood bilirubin. *Biochem. Zeitschrift.* **297** : 81-89.

Jimoh, A. O.; Shaibu, O. B.; Emmanuel, U. E.; Solomon, A. A. and Vincent, U. I. (2011). Comparative Pharmacokinetics of Intramuscular Ceftriaxone Co-Administered with Acetaminophen in Healthy and Infected Sokoto Red Goats. *Int. J. Pharm.* **7**: *623-628.*

Johri, R. K. and Zutshi, U. (1992). Ayurvedic formulation 'Trikatu' and its constituents. J Ethnopharmacol. *J. Ethanopharmcol*. **37**: 85-91.

Joly, V.; Pangon, B.; Brion, N.; Vallois, J. and Carbon, C. (1988). Enhancement of the therapeutic effect of cephalosporins in experimental endocarditis by altering their pharmacokinetics with diclofenac. *J. Pharmacol. & Exper. Therap*. **246(2):** 695-700.

Jones, R. N. (1998). Important and emerging -lactamase-mediated resistances in hospital-based pathogens: the AmpC enzymes. *Diagn. Microbiol. Infect. Dis*.; **31**: 461-466.

Jones, R. N.; Pfaller, M. A.; Men, S. D.; Gerlach, E. H.; Fuchs, P. C. and Aldridge, K. E. (1991). Antimicrobial activity of cefpirome. An update compared to third-generation cephalosporins against nearly 6000 recent clinical isolates from five medlcal centres. *Diagn. Microbiol. Infect. Dis.* **14:** 361-364.

Jun, R.; Jun-ren, Z.; Zu-gong, Y. and Shan-xiang, J. (2008). Study on Acute Toxicity and Cumulative Toxicity of Domestic Cefquinome Sulfate in Mice. Progress in Veterinary Medicine. pp: 02.

Kadota, T.; Kondoh, H.; Chikazawa, H.; Kawano, S.; Kuroyanagi, K.; Ohta, S.; Ishikawa, K.; Takahashi, N.; Kohmura, H. and Kai, S. (1990). Four week repeated dose oral toxicity study of BMY-28142 (cefepime) in juvenile rats. *Jpn. J. Antobiot.* **43(7):** 1243-1259.

Kadota, T.; Kondoh, H.; Chikazawa, H.; Kuroyanagi, K.; Ishikawa, K.; Kawano, S.; Sakakura, K.; Takahashi, N.; Funahashi, N.; and Shimizu, N. (1992). Cefepime (diHCl/L-arginine blend): intravenous continuous infusion and/or single dose subcutaneous toxicity study in rats and dogs. *Jpn. J Antibiot.* **45(6)**:612-9.

Kai, S.; Kohmura, H.; Ishikawa, K.; Kawano, S.; Sakai, A.; Kuroyanagi, K.; Kadota, T. and Takahashi, N. (1992). Reproductive and developmental toxicity studies on cefepime dihydrochloride administered subcutaneously to rats during the premating, gestation and lactation periods. *Jpn. J. Antibiot.* **45(6)** : 642-60.

Kalman, D. and Barriere, S. L. (1990). Review of the pharmacology, pharmacokinetics, and clinical use of cephalosporins. *Texas heart institute J.* **13(3):** 203-215.

Kang, M. H.; Won, S. M.; Park, S. S.; Kim, S. G.; Novak, R. F. and Kim, N. D. (1994). Piperine effects on the expression of P4502E1, P4502B and P4501A in rat. *Xenobiotica*. **24**(12):1195-1204.

Karan, R. S.; Bhargava, V. K. and Garg, S. K. (1998). Effect of trikatu (piperine) on the pharmacokinetic profile of isoniazid in rabbits. *Indian J. Pharmacol.* 30(**4**): 254-256.

Karan, R. S.; Bhargava, V. K. and Garg, S. K. (1999[a]). Effect of Trikatu on the pharmacokinetic profile of Indomethacin in rabbits. *Indian J. Pharm.* 31:160-161.

Karan, R. S.; Bhargava, V. K. and Garg, S. K. (1999[b]). Effect of trikatu, an Ayurvedic prescription, on the pharmacokinetic profile of rifampicin in rabbits. *J. Ethnopharmacol.* **64(3)**: 259-264.

Khajuria, A.; Zutshi, U. and Bedi, K.L. (1998). Permeability characteristics of piperine on oral absorption an active alkaloid from peppers and a bioavailability enhancer. *Indian J. Exp. Biol*. **36**(1): 46-50.

Kikuchi, N.; Kagota, C.; Nomura, T.; Hiramune, T.; Takahashi, T. and Yanagawa, R. (1995). Plasmid profiles of *Klebsiella pneumoniae* isolated from bovine mastitis. *Vet. Microbiol*. **47**: 9-15.

Kim, K. S. and Bayer, A. S. (1985). Efficacy of BMY-28142 in experimental bacteremia and meningitis cause by E coli and group B streptococci. *Anti. Microb. Agent. Chemother.* **28(1):** 51-54.

Kollef, M. H. (2009). New antimicrobial agents for methicillin-resistant *Staphylococcus aureus. Crit. Care Resusc.* **11(4)**:282-6.

Kumar, N.; Singh S.D. and Jayachandran C. (2003). Pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin and its interaction with diclofenac after intravenous administration in buffalo calves. *The Vet. J.* **165**: 302–306.

Kumar, U.; Sinha, S. P.; Jayachandran, C. (2002). Comparative pharmacokinetics and dosage regimen of enrofloxacin in febrile and afebrile goats. *Indian J. Anim. Sci.* **72**: 954-956.

Lamb, L. M.; Hibbard, J.R.; Desiderio, J. V. and Tsai, Y.H. (1993). Efficacy of cefepime in a Staphylococcus aureus endocarditis rat model. *J. Antimicrob. Chemother.* **32:** 95-101.

Landoni, M. F. and Lees, P. (1995). Influence of formulation on pharmacokinetic and bioavailability of racemic ketoprofen in horse. *J. Vet. Pharmacol. Ther.* **18:**446-450.

Landoni, M. F; Cunningham, F. M. and Lees, P. (1996[a]). Pharmacokinetics and pharmacodynamics of tolfenamic acid in calves. *Research in Veterinary Science*. **61:** 26-32.

Landoni, M.F; Foot, R; Frean, S. and Lees, P. (1996[b]). Effects of flunixin, tolfenamic acid, R(-) and S(+) ketoprofen on the response of equine synoviocytes to lipopolysaccharide stimulation. *Equine Veterinary Journal*. **28:**468–475.

Lees, P; Landoni, M.F; Giraudel, J. and Toutain, P.L. (2003). Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *J. Vet. Pharmacol. Ther.* **27:**479–490.

Lees, P; McKellar, Q. A; Foot, R. and Gettinby, G. (1998). Pharmacodynamics and pharmacokinetics of tolfenamic acid in ruminating calves: evaluation in models of acute inflammation. *The Veterinary Journal.* **155:**275–288.

Leive, L. (1974). The barrier function of the gram-negative envelope. *Acad. Sci.* **5**: 109-111.

Leophonte, P.; Bertrand, A.; Nouvet, G.; Muir, J. F.; Lucht, F.; Delaval, P.; Depierre, A.; Hughes, F.; Ulmer, M. and Gress, J. J. (1993). A comparative study of cefepime and ceftazidime in the treatment of community-acquired lower respiratory tract infections. *J. Antimicrob. Chemother.* **32(1):** 165-173.

Li, X. B.; Wu, W. X.; Su, D.; Wang, Z. J.; Jiang, H. Y. and Shen, J. Z. (2008). Pharmacokinetics and bioavailability of cefquinome in healthy piglets. *J. Vet. Pharmacol. Therap.* **31**: 523–527.

Liguo, Y.; Jian, S.; Rui, W; Lihua, S.; Lixiang, Z.; Xianyang, L.; Binghu, Fang. and Yahong, L. (2011). Pharmacokinetics and bioavailability of cefquinome in healthy ducks. *Am. J. Vet. Res.* **72(1)**: 122-126.

Limbert, M.; Isert, D.; Klesel, D.; Markus, A.; Seeger, K.; Seibert, G. and Schrinner, E. (1991). Antibacterial activity in-vitro and in-vivo and pharmacokinetics of cefquinome (HR 111V), a new broad spectrum cephalosporin. *Antimicrobial Agents and Chemotherapy.* **35(1):**14-19.

Linares, J.; Alonso, T. and Perez, J. L. (1992). Decreased susceptibility of penicillin-resistant pineumococci to twenty-four beta-lactam antibiotics. *J. Antiniicrob. Chemother*. **30:** 279-88.

Linden, I. B.; Parantainen, J.; Karppanen, H. and Vapaatalo, H. (1975). Inhibitory effects of tolfenamic acid, indomethacin and acetylsalicylic acid on prostaglandin synthetase and phospho-diesterase *in vitro*. In: *Sixth International Congress of Pharmacology*, Helsinki, Finland. July 20–25, Abstract 594.

Linden, I. B; Parantainen, J. and Vapaatalo, H. (1976). Inhibition of prostaglandin biosynthesis by tolfenamic acid in vitro. *Scandinavian Journal of Rheumatology*. **5:**129–132.

Livermore, D.M. and Williams, J.D. (1996). -Lactams: Mode of Action and Mechanisms of Bacterial Resistance. In Antibiotics In Laboratory Medicine; Lorian, V. Ed.; Williams & Wilkins: Philadelphia, pp 502-578.

Lu, G. F.; Yang, H. F.; Li, Y. J. and Jiang, C. M. (2007). Pharmacokinetics of cefquinome sulfate suspension in pigs. *Journal of Yangzhou University*. **28**:18–20.

Maden, M.; Tra , B.; Ba , A. L.; Elmas, M.; Yazar, E. and Birdane, F. M. (2001). Investigation of biochemical and haematological side-effects of cefquinome in healthy dogs. *Veterinary Quarterly*. **23(1)**: 32-34.

Maes, A.; Meyns, T.; Sustronck, B.; Maes, D.; De Backer, P. and Croubels, S. (2007). Determination of cefquinome in pig plasma and bronchoalveolar lavage fluid by high-performance liquid chromatography combined with electrospray ionization mass spectrometry. *J. Mass spectrom.* **42 (5):**657-63

Majumdar, A.M.; Dhuley, J.N.; Deshmukh, V.K. and Naik, S.R. (1999). Effect of Piperine Bioavailability of Oxyphenylbutazone in rats. *Indian Drugs*. **36**:123.

Majumdar, A.M.; Dhuley, J.N.; Deshmukh, V.K.; Raman, P.H. and Naik, S.R. (1990[a]). Antiinflammatory activity of piperine, *Japan J. Med Sc Biol.* **43**: 95.

Majumdar, A.M.; Dhuley, J.N.; Deshmukh, V.K.; Raman, P.H.; Tharat, S.L. and Naik, S.R. (1990[b]). Effect of piperine on pentobarbitore induced hypnosis in rats. *Indian. J. Exp. Biol.* **28**:486.

Mandell, G. L. and Petri, W. A. Jr. (1996). Antimicrobial agents: Penicillins, Cephalosporins and Other -lactam Antibiotics. In Hardman, J.G. and Limbird, L. E. (Eds.). Goodman and Gillman's "The pharmacological basis of therapeutics". 9th Edn. McGraw Hill, NY. pp. 1073-1101.

Marchou, B.; Michea, H. M.; Lucain, C. and Pechere, J. C. (1987). Development of -lactam-resistant *Enterobacter cloacae* in mice. *J. Infec. Dis.* **156(2):** 369-373.

Mathur, P.; Velpandium ,T.; Sengupta, S. and Gupta, S.K. (1998). Effect of piperine on analgesic activity of Nimesulide: A possible pharmacokinetic interaction. *Indian J. Pharm.* **30**:204.

McKellar, Q. A; Galbraith, E. and Simmons, R. (1991). Pharmacokinetics and serum thromboxane inhibition of two NSAIDs when administered to dogs by the intravenous or subcutaneous route. *Journal of Small Animal Practice*. **32:**335-340.

McKellar, Q. A; Lees, P. and Gettinby, G. (1994). Pharmacodynamics of tolfenamic acid in dogs. Evaluation of dose response relationship. *European Journal of Pharmacology*. **253:**191–200.

Merck (2000). General information on piperine. In: Merck index, Germany, pp: 1030.

Mimoz, O.; Elhelali, N.; Leotard, S.; Jacolot, A.; Laurent, F.; Samii, K.; Peitjean, O. and Nordmann, P. (1999). Treatment of experimental pneumonia in rats caused by a PER-1 extended-spectrum -lactamase-producing strain of *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* **44(1):** 91-97.

Mimoz, O.; Jacolot, A.; Padoin, C.; Caillon, J.; Louchahi, K.; Tod, M.; Samii. K. and Petitjean, O. (1997). Cefepime and amikacin synergy against a cefotaxime-susceptible strain of *Enterobacter cloacae in-vitro* and *in-vivo. J. Antimicrob. Chemother.* **39(3):** 363–369.

Murphy, S. P.; Erwin, M. E. and Jones, R.N. (1994). Cefquinome (HR IIIV), *in vitro* evaluation of a broad spectrum cephalosporin indicated for infection in animals. *Diagn. Microbiol. Infect Dis*. **20**: 49-55.

Murray, B. E. and Moellering, R. C. (1978). Patterns and mechanisms of antibiotic resistance. *Med. Clin. North. Am.* **62**: 899-923.

Naito, T.; Aburaki, S.; Kamachi, H.; Narita, Y.; Okumura, J. and Kawaguchi H. (1986). Synthesis and structure-activity relationships of a new series of cephalosporins, BMY-28142 and related compounds. *J. Antibiot.* **39(8)**:1092–1107.

Nduka, S. O.; Okonta, M. J. and Esimone, C. O. (2013). Effects of Zingiber officinale on the Plasma Pharmacokinetics and Lung Penetrations of Ciprofloxacin and Isoniazid. *Am. J. Ther.* **20(5)**: 495-590

Neu, H. C. (1982). Mechanisms of bacterial resistance to antimicrobial agents, with particular reference to cefotaxim and other -lactam compounds. *Rev. Infec.Dis.* **4**(suppl.): 288-299.

Norrby, S. R. (1987). Side effects of cephalosporins: a review. *Drugs.* **34(2):** 105-120.

Norrby, S.R. (1993). Cefpirome: Efficacy in the treatment of urinary and respiratory tract infections and safety profile. *Scand. J. Infect. Dis.* **91:**41-50.

Ogino, T.; Mizuno, Y.; Ogata, T. and Takahashi, Y. (2005). Pharmacokinetic interactions of flunixin meglumine and enrofloxacin in dogs. *Am. J. Vet. Res.* **66**(7): 1209-1213.

Okamoto, M.P.; Nakahiro, R.K.; Chin, A.; Bedikian, A. and Gill, M.A. (1994). Cefepime: a new fourth-generation cephalosporin. *Am. J. Hosp. Pharm.* **51(4):** 463-477.

Palanivel, K. M.; Selvasubramanian, S. and Nedunchelliyan, S. (2005). Treatment of clinical mastitis with Cefquinome. *Indian Vet. J.* **82(12)**:1313-1314.

Papich, M. G. (1984). The beta-lactam antibiotics: Clinical pharmacology and recent developments. *Compend. Contin. Educ. Pract. Vet.* **9**(1): 68-75. Cited by Swati, 2004.

Papich, M. G. (1987). Clinical pharmacology of cephalosporin antibiotics. J. Am. Vet. Med. Assoc. **184**(3): 344-347.

Patel, (2012). Studies on effect of ketoprofen, febrile condition and bioenhancer trikatu and its constituents on pharmacokinetics of levofloxacin and safety of

levofloxacin in goats. Ph.D. Thesis. Anand Agricultural University. Gujarat, India.

Patel, H. B.; Patel, N. N.; Patel, S. D.; Dewda, S.; Patel, J. H.; Bhavsar, S. K. and Thaker, A. M. (2012[a]). Effect of Ketoprofen Co-administration and Febrile State on Pharmacokinetic of Cefepime in Goats. *Asian Journal of Animal and Veterinary Advances,* **7**: 46-53.

Patel, N. N.; Patel, H. B.; Patel, S. D.; Dewda, S.; Patel, J. H.; Bhavsar, S. K. and Thaker, A. M. (2012[b]). Effect of Ketoprofen Co-administration or Febrile State on Pharmacokinetic of Cefepime in Sheep. *Veterinarski. Arhiv.* **82** (5), 473-481.

Patel, S. D.; Sadariya, K. A.; Gothi, A. K.; Patel, U. D., Jain, M. R.; Bhavsar, S. K. and Thaker, A. M. (2011[a]). Safety of tolfenamic acid following repeated intramuscular administration in wistar rats. *Pharma Sci. Monitor Int. J. Pharm. Sci.*, **2**: 79-85.

Patel, S.; Devada, S.; Patel, H.; Patel, N.; Bhavsar, S. and Thaker, A. (2011[b]). Influence of Co-Administration of Piperine on Pharmacokinetic Profile of Gatifloxacin in Layer Birds. *Global Veterinaria* **7(5):** 427-432.

Patel, U. D. (2009[b]). *Studies on effect of ketoprofen and febrile condition on pharmacokinetics of levofloxacin and safety of levofloxacin alone and in combination with ketoprofen in sheep*. M.V.Sc. Thesis. Anand Agricultural University. Gujarat, India.

Patil, A. J. (2010). Studies on effect of fever and co-adminstration of ketoprofen on pharmacokinetics of cefepime and safety of repeated simultaneous intramuscular administration of cefepime and ketoprofen in cow calves. M.V.Sc. Thesis. Anand Agricultural University, Anand.

Patwardhan, B. and Vaidya, A. D. (2010). Natural products discovery: Accelerating the clinical candidate development using reverse pharmacology approaches. *Ind. J. Exp. Biol*. **48** : 220–227.

Pei, Y. Q. (1983). A review of pharmacology and clinical use of piperine and its derivatives. *Epilepsia*. **24**(2):177-182.

Pentikainen, P. J; Neuvonen, P. and Backman, C. (1981). Human pharmacokinetics of tolfenamic acid, a new anti-inflammatory agent. *European Journal of Clinical Pharmacology*. **19:**359-365.

Petri, W. A. (2001). Antimicrobial agents: Penicillins, Cephalosporins and Other β-lactam Antibiotics. In Hardman, J.G. and Limbird, L.E. (eds.). Goodman and Gillman's "The pharmacological basis of therapeutics". 10th Edn. McGraw Hill, NY, pp. 1189-1218.

Phelps, D. J.; Carlton, D. D.; Farrell, C. A. and Kessler, R. E. (1986). Affinity of cephalosporins for -lactamases as a factor in antibacterial efficacy. *Antimicrob. Agents Chemother.* **29(5):** 845-848.

Platel, K. and Srinivasan, K. (2001). Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung*. **44**(1)**:**42-46.

Prescott, J. F. and Baggot, J. D. (1993). Beta-lactam antibiotics: Cephalosporins andcephamycin. In: Antibecterial therapy in Veterinary, Second edn. International Book Distributing Company, Lucknow, India, PP. 98-118.

Preston, D. A. (1992). Overview of the development of a new class of -lactam antibiotics: The carbacephems. *Antimicrobic Newslett*. **8**: 58-63.

Pucci, M. J.; Boice-Sowek, J.; Kessler, R. E. and Dougherty, T. J. (1991). Comparison of cefepime, cefpirome and cefaclidine binding affinity for

penicillin-binding proteins in *Escherichia coli* K-12 and *Pseudomonas aeruginosa* SC 8329. *Antimicrob. Agents Chemother.* **35(11):** 2312-2317.

Quin, J. D. (1989). The nephrotoxicity of cephalosporins. *Adverse Drug React. Acute Poisioning Rev.* **8(2):** 63-72.

Rahal, A; Amit Kumar; Ahmad, A.H. and Malik, J.K. (2008). Pharmacokinetics of diclofenac and its interaction with enrofloxacin in sheep. *Res. Vet. Sci.* **84**: 452–456.

Raj, K. P. S. and Nagarsheth, H. K. (1978). “Pepper - A Review article,” *Indian Drugs* .**16 (1)**: 199- 203.

Rankin, G. O. and Sutherland, C. H. (1989). Nephrotoxicity of aminoglycosides and cephalosporins in combination. *Adverse Drug React. Acute Poisioning Rev.* **8(2):** 73-88.

Rao; G.S.; Ramesh; S.; Ahmad, A.H.; Tripathi, H.C.; Sharma, L.D. and Malik, J.K. (2000). Effects of endotoxin-induced fever and probenecid on disposition of enrofloxacin and its metabolite ciprofloxacin after intravascular administration of enrofloxacin in goats. *J. Vet. Pharmacol. Ther*. 23: 365–372.

Reen, R. K. and Singh, J. (1991). *In vitro* and *In vivo* inhibition of pulmonary cytochrome P450 activities by piperine, a major ingredient of piper species. *Indian J. Exp. Biol.* **29**(6): 568-573.

Reiffers, J.; Makhoul, C.; Pris, J. (1992). Efficacy of cefpirome in the initial empiric management of febrile mahgnancies. Program and Abstracts of the 32nd Inteecience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, California. Abs 1575.

Robertson, S. A. and Taylor, P. M. (2004). Pain management in cats-past, present and future. Part 2. Treatment of pain-clinical pharmacology. *Journal of Feline Medical Surgery*. **6:**321–333

Rolinson, G.N. (1986). -lactam antibiotics. *J. Antimicrob. Chemother.* **17(1)**: 5-36.

Sader, H. S. and Jones, R. N. (1993). The fourth-generation cephalosporins: antimicrobial activity and spectrum definitions using cefpirome as an example. *Antimicrob.Newsl.* **9**:9–16.

Salyers, A. A. and Dixie, D. W. (2002). Bacterial Pathogenesis: A Molecular Approach. Washington: ASM Press. Cited by Swati, 2004.

Sanders, W.E. and Sanders, C.C. (1988). Inducible -lactamases: clinical and epidemiologic implications for use newer cephalosporins. *Rev. Infect. Dis.* **10(4):** 830-838.

Schafer, V.; Shah, P.M.; Doerr, H.W.; Ziemen, M.; Hellwich, S. and Seibert, G. (1992). In-vitro activity of cefpirome against isolates from patients with urinary tract, lower respiratory tract and wound infections. J. Antimicrob. Chemother. pp. 7-12.

Sharma, A. K. and Varshneya, C. (2009). Pharmacokinetics of enrofloxacin following oral administration in pre-ruminant cow calves and its modulation by pretreatment of Trikatu, a herbal bio-enhancer. In: Compedium of IXth Annual conference of Indian society of veterinary pharmacology and toxicology 5-7 november. pp: 249

Sharma, K. K.; Sangraula, H. and Mediratta, P. K. (2002). Some new concepts in antibacterial drug therapy. *Ind. J. Pharmacol*. **34(6)**: 390-396.

Shobha, G.; Joseph, T.; Majeed, M.; Rajendran, R. and Srinivas, P. S. S. R. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers, *Plant Med.* **64**: 353.

Shpigel, N. Y. and Schmid, P. (1997). Contribution to the treatment of acute bovine mastitis with cefquinome. *Tierarztl Prax*. **25**: 200-206.

Shpigel, N. Y.; Levin, D.; Winkler, M.; Saran, A.; Ziv, G. and Bottner, A. (1997). Efficacy of cefquinome for treatment of cows with mastitis experimentally induced using Escherichia coli. *J. Dairy Sci.* **80**:318–323.

Sidhu, P. K; Landoni, M. F. and Lees, P. (2005). Influence of marbofloxacin on the pharmacokinetics and pharmacodynamics of tolfenamic acid in calves. *J. Vet. Pharmacol. Ther.* **28(1):**109-119.

Singh, J.; Dubey, R. K. and Atal, C. K. (1986). Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: evidence that piperine lowers the endogeneous UDP-glucuronic acid content. *J. Pharmacol. Exp. Ther.* **236(2):** 488-493.

Singh, M.; Varshneya, C.; Telang, R. S. and Srivastava A. K. (2005). Alteration of pharmacokinetics of oxytetracycline following oral administration of Piper longum in hens. *J. Vet. Sci.* 6(3):197–200.

Singh, R.; Chaudhary, R. K. and Dumka, V. K. (2008). Influence of paracetamol on the pharmacokinetics and dosage regimen of ceftizoxime in cross bred calves. *Isra. J. Vet. Med*. **63**: 72-76.

Singh, R.; Devi, S.; Patel, J. H.; Patel, U. D.; Bhavsar, S. K. and Thaker, A. M. (2009). Indian Herbal Bioenhancers: A Review. *Phcog. Rev.* **3(5)**: 90-92.

Singh, T. B.; Kumar, N. and Jayachandran, C. (2013). Pharmacokinetics of diclofenac and its interaction with cefotaxime in female goats after intravenous

administration. *International Journal of Current Pharmaceutical Research.* **3(4)**:91-98.

Slingsby, L.S. and Waterman-Pearson, A.D. (2000). Postoperative analgesia in the cat after ovariohysterectomy by use of carprofen, ketoprofen, meloxicam or tolfenamic acid. *Journal of Small Animal Practice.* **41:**447–450.

Smiet, E.; Haritova, A.; Heil, B. A.; Fink-Gremmels, J. and Wijnberg, I. D. (2012). Comparing the pharmacokinetics of a fourth generation cephalosporin in three different age groups of New Forest ponies. *Equine Vet. J. Suppl.* **41**:52-6.

Song, I. B.; Kim, T. W.; Lee, H. G.; Kim, M. S.; Hwang, Y. H.; Park, B. K.; Lim, J. H. and Yun, H. I. (2013). Influence of the Injection Site on the Pharmacokinetics of Cefquinome Following Intramuscular Injection in Piglets. *J. Vet. Med. Sci.* **75(1)**:89–92.

Sorensen, L. K. and Snor, L. K. (2000). Determination of cephalosporins in raw bovine milk by high-performance liquid chromatography. *Journal of Chromatography A*. (882):145–151

Spencer, R. C.; Bauernfeind, A. and Garcia-Rodriguez, J. (1997). Surveillance of current resistance of nosocomial pathogens to antibiotics. *Clinical Microbiology and Infection*. **3(1):** 87-101.

Spratt, B.G. (1994). Resistance to antibiotics mediated by target alterations. *Science* **264**: 388-393.

Sultana, N.; Arayne, M. S. and Afzal, M. (2003). Synthesis and antibacterial activity of cephradine metal complexes: part I complexes with magnesium, calcium, chromium and manganese. *Pak. J. Pharm. Sci.* **16(1):** 59-72.

Sultana, N.; Arayne, M. S. and Afzal, M. (2005). Synthesis and antibacterial activity of cephradine metal complexes: part II complexes with cobalt, copper, zinc and cadmium. *Pak. J. Pharm. Sci.* **18(1):** 36-42.

Sweet, R. L.; Gibbs, R. S. (2009). Infectious Diseases of the Female Genital Tract. *Lippincott Williams & Wilkins*. pp. 403.

Sweetman, S. C. (2002). In: Martindale: The Complete Drug Reference, 33rd Ed., The Pharmaceutical Press, London, UK. pp: 47-48.

Talke, H. and Schubert, G. E. (1965). Enzymatic urea determination in the blood and serum in the Warburg optical test. *Klinische Wochenschrift.* 43: 174-175.

Tally, F. P.; McGowan, K.; Kellum, J. M.; Gorbach, S. L. and O'Donnell, T. F. (1981). A randomized comparison of cefoxitin with or without amikacin and clindamicin plus amikacin in surgical sepsis. *Ann. Surg.* **193(3):** 318-323.

Thomas, E.; Martin, G.; Voss, B.; Boettner, A.; Pommier, P.; Loehlein, W. and Hellmann, K. (2002). A comparative field study of the efficacy of cefquinome and amoxycillin against acute meningitis of weaned piglets. *The pig Journal* **50**:28-41.

Thomas, E.; Thomas, V. and Wilhelm, C. (2006). Antibacterial activity of cefquinome against equine bacterial pathogens. *Vet. Microbiol.* **115:**140-147.

Thomas, J. A. and Burns, R. A. (1998). Important Drug- Nutrient Interactions in the Elderly. *Drugs & Aging*. **13(3):** 199-209.

Thomson, T. D.; Quay J. F. and Webber, J. A. (1984). Cephalosporin group of antimicrobial drugs. *J. Am. Vet. Med. Assoc.* **185**(10): 1109-1114.

Tietz, N. W. (1987). Fundamentals of Clinical Chemistry, 3rd Edn. W. B. Saunders, Philadelphia, USA.

Tietz, N. W. (1995). Clinical Guide to Laboratory Test, 3rd Edn. Philadelphia, Pa: W. B. Saunders

Tohamy, M. A. (2011). Age-related intramuscular pharmacokinetics of cefquinome in sheep. *Small Ruminant Res.* **99**:72–76.

Tohamy, M. A.; Ismail, M.; and El- Gendy, A. A. M. (2006). Comparative pharmacokinetics of cefquinome in ruminants. *J. Egypt. Soc. Pharmacol. Exp. Ther.*, **4**: 12-18.

Tomasz, A. (1979). The mechanism of the irreversible antimicrobial effects of penicillins. *Ann. Rev. Microbiol.* **33**: 113-37.

Tsai, Y. H.; Bias, M.; Leitner, F. and Kessler R. E. (1990). Therapeutic studies of cefepime (BMY-28142) in murine meningitis and pharmacokinetics in neonatal rats. *Antimicrob. Agents Chemother.* **34(5):** 733-738.

Uney, K.; Altan, F. and Elmas, M. (2011). Development and validation of a high-performance liquid chromatography method for determination of cefquinome concentrations in sheep plasma and its application to pharmacokinetic studies. *Antimicrob. Agents Chemother*. **55(2)**:854-859.

Vaden, S. L. and Riviere, J. E. (2001). Penicillins and related β-lactam antibiotics. In "Veterinary Pharmacology and Therapeutics" Adams, H.R. (ed.), 8th Edn.; 2001. Iowa State University Press, Ames, IA. pp. 818-827.

Vane, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature*. **231:**232–235.

Varma A.R.; Ahmad, A.H. and Sharma, LD. (2000). Pharmacokinetics of enrofloxacin and its interaction with diclofenac sodium in cattle. In: Compendium of First National Annual Conference of Indian Society of

Veterinary Pharmacology and Toxicology, December 6–8, 2000 Ludhiana, Punjab. pp: 46.

Verma, D.K. and Roy, B.K. (2006). Milk kinetics of gatifloxacin after single dose intravenous administration in healthy and febrile goats. *Indian J. Pharmacol.* **38**: 366-367.

Walunj, (2008). Studies on effect of bioenhancer trikatu on pharmacokinetics of gatifloxacin and safety of gatifloxacin in sheep. M.V.Sc. Thesis. Anand Agricultural University. Gujarat, India.

Wasfi, I. A; Elghazali, M; Hadi, A. A, Zorob, O; Boni, N. S; Alkatheeri, N. A. and Barezaiq I. M. (1998). Pharmacokinetics of tolfenamic acid and its detection time in urine after intravenous administration of the drug in camels. *Am J Vet Res*. **59(11):**1451-1458.

Waxman, S.; SanAndres, M.D.; Gonzalez, F.; DeLucas, J.J.; SanAndres, M.I. and Rodrıguez, C. (2003). Influence of *Escherichia coli* endotoxin-induced fever on the pharmacokinetic behavior of marbofloxacin after intravenous administration in goats. *J. Vet. Pharmacol. Ther.* **26**:65-69.

Widmer, A.; Kummer, M.; Eser, M. W. and Furst, A. (2009). Comparison of the clinical efficacy of cefquinome with the combination of penicillin G and gentamicin in equine patients. *Equine Veterinary Education.* **21** (**8**): 430-435.

Wilson, D. J.; Gonzalez, R. N. and Das, H. H. (1996). Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects of somatic cell count and milk production. *J. Dairy Res*. **80**: 2592-2598.

Winther, L.; Baptiste, K. E. and Friis, C. (2011). Antimicrobial disposition in pulmonary epithelial lining Fluid of horses, PartIII. Cefquinome. *J. Vet. Pharmacol. Ther*. **34**:482–486.

Xie, W.; Zhang, X.; Wang, T. and Du, S. (2013). Pharmacokinetic analysis of cefquinome in healthy chickens, *British Poultry Science.* **54(1)**: 81-86.

Yang, D. W.; Chen, Z. L.; Ding, H. Z.; Shen, X. G.; Xu, S. S. and Gu, X. Y. (2009). Pharmacokinetics and bioavailability of cefquinome in pigs. *Chinese Journal of Veterinary Science.* **29 (9)**: 1182-1185.

Yuan, L.; Sun, J.; Wang, R.; Sun, L.; Zhu, L.; Luo, X.; Fang, B. and Liu, Y. (2011). Pharmacokinetics and bioavailability of cefquinome in healthy ducks. *Am. J. Vet. Res.* **72(1)**:122-126.

Zonca, A.; Gallo, M.; Locatelli, C.; Carli, S.; Moroni, P.; Villa, R. and Cagnardi, P. (2011). Cefquinome sulfatebehavior after intramammary administration in healthy and infected cows. *J. Dairy. Sci.* **94(7)**:7.

Zutshi, R. K.; Singh, R.; Zutshi, U.; Johri, R. K. and Atal, C. K. (1985). Influence of piperine on rifampicin blood levels in patients of pulmonary tuberculosis. *J. Assoc. Physicians India.* **33**:223-224.

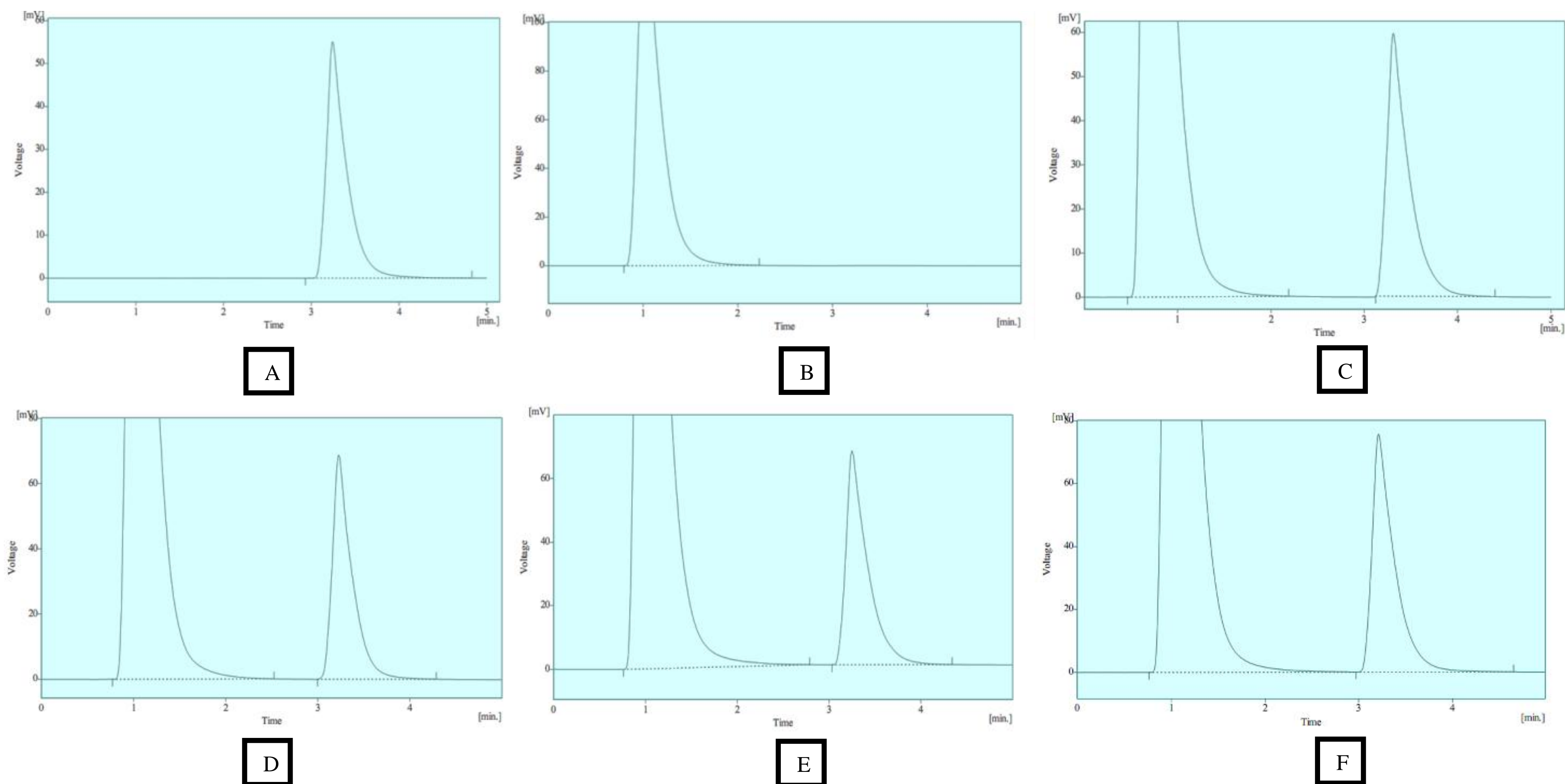

Figure 4.1: Representative chromatograms of A) Cefquinome (2.5 µg/mL) in mobile phase, B) Blank plasma of sheep, C) Cefquinome (2.5 µg/mL) in plasma of sheep D) 1 h post intramuscular administration of cefquinome alone E) 1 h post intramuscular administration of cefquinome in combination with tolfenamic acid F) 1 h post intramuscular administration of cefquinome in trikatu pretreated sheep.

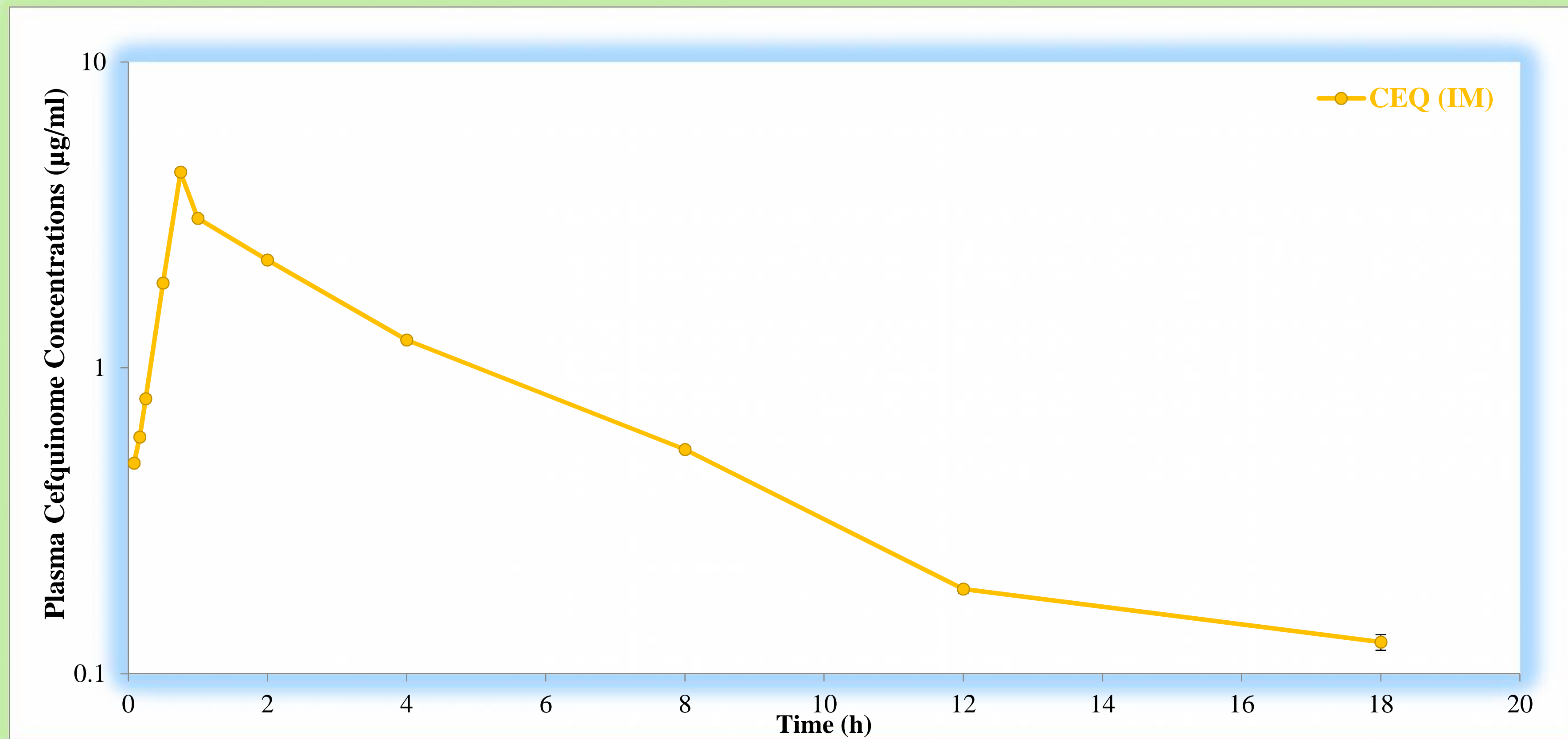

Figure 4.2: Semi logarithmic plot of cefquinome concentration in plasma versus time following single dose intramuscular administration at the dose rate of 2 mg/kg in healthy sheep. Each point represents mean ± SE of six animals.

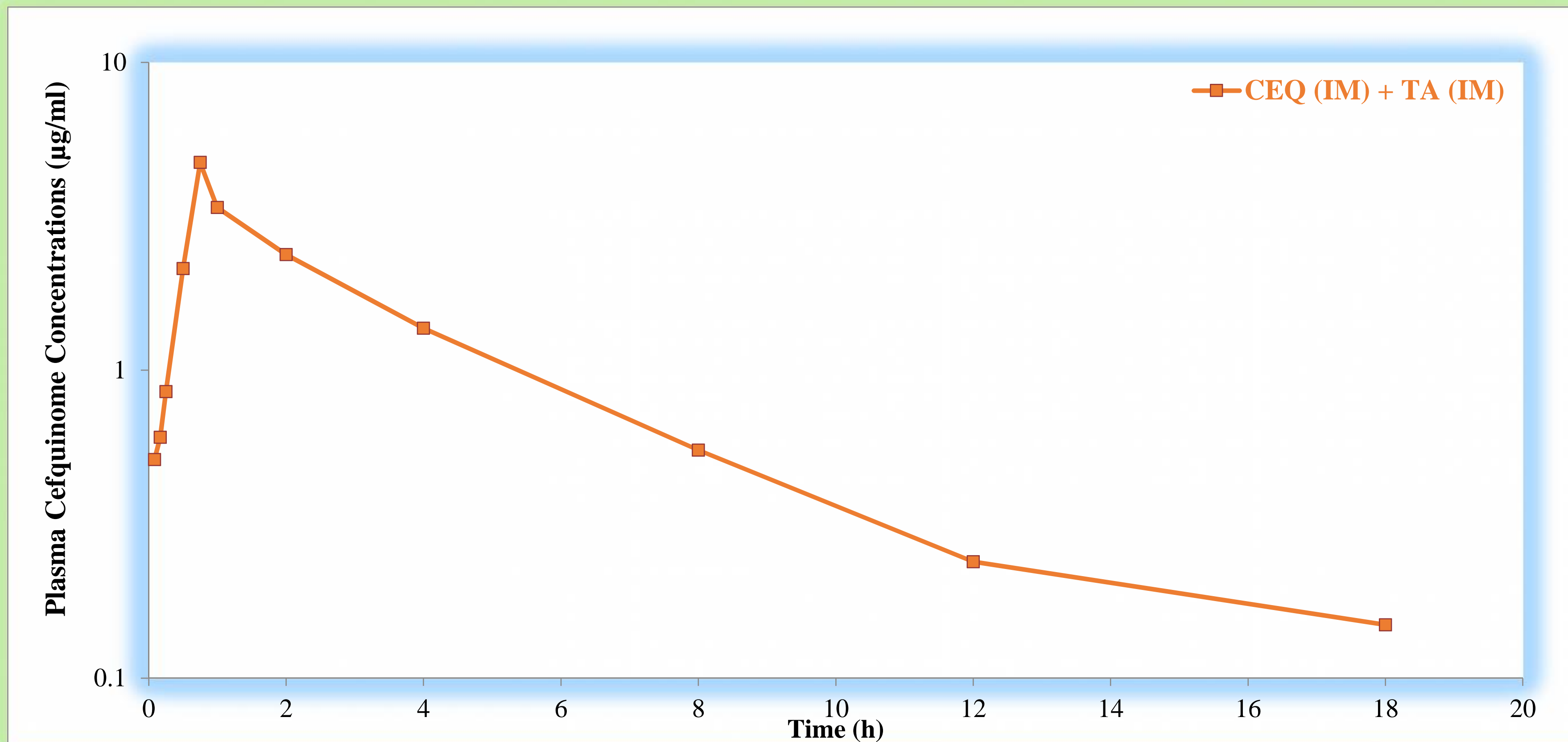

Figure 4.3: Semi logarithmic plot of cefquinome concentration in plasma versus time following single dose intramuscular administration at the dose rate of 2 mg/kg in tolfenamic acid (2 mg/kg) treated sheep. Each point represents mean ± SE of six animals.

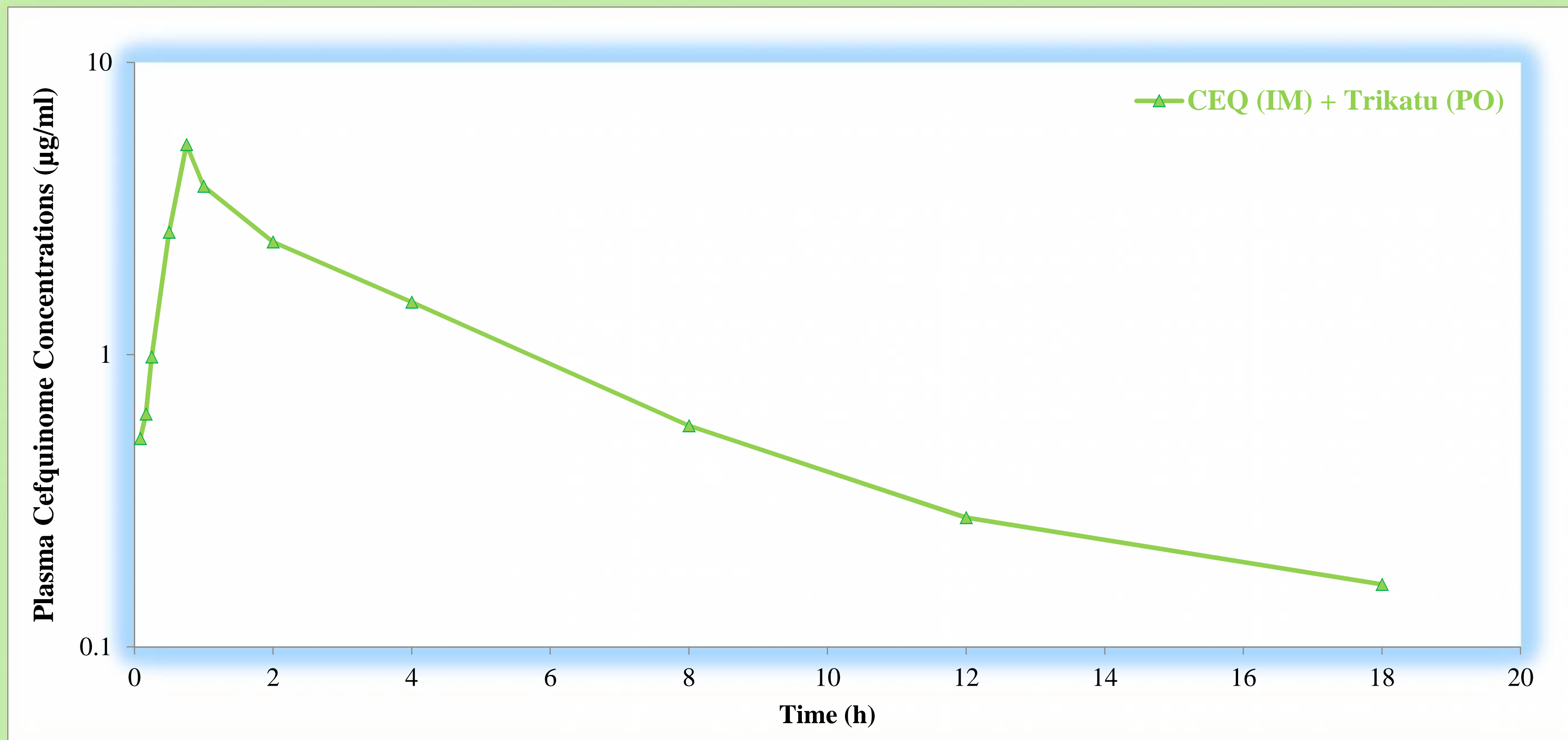

Figure 4.4: Semi logarithmic plot of cefquinome concentration in plasma versus time following intramuscular administration at the dose rate of 2 mg/kg in trikatu pretreated sheep. Each point represents mean ± SE of six animals.

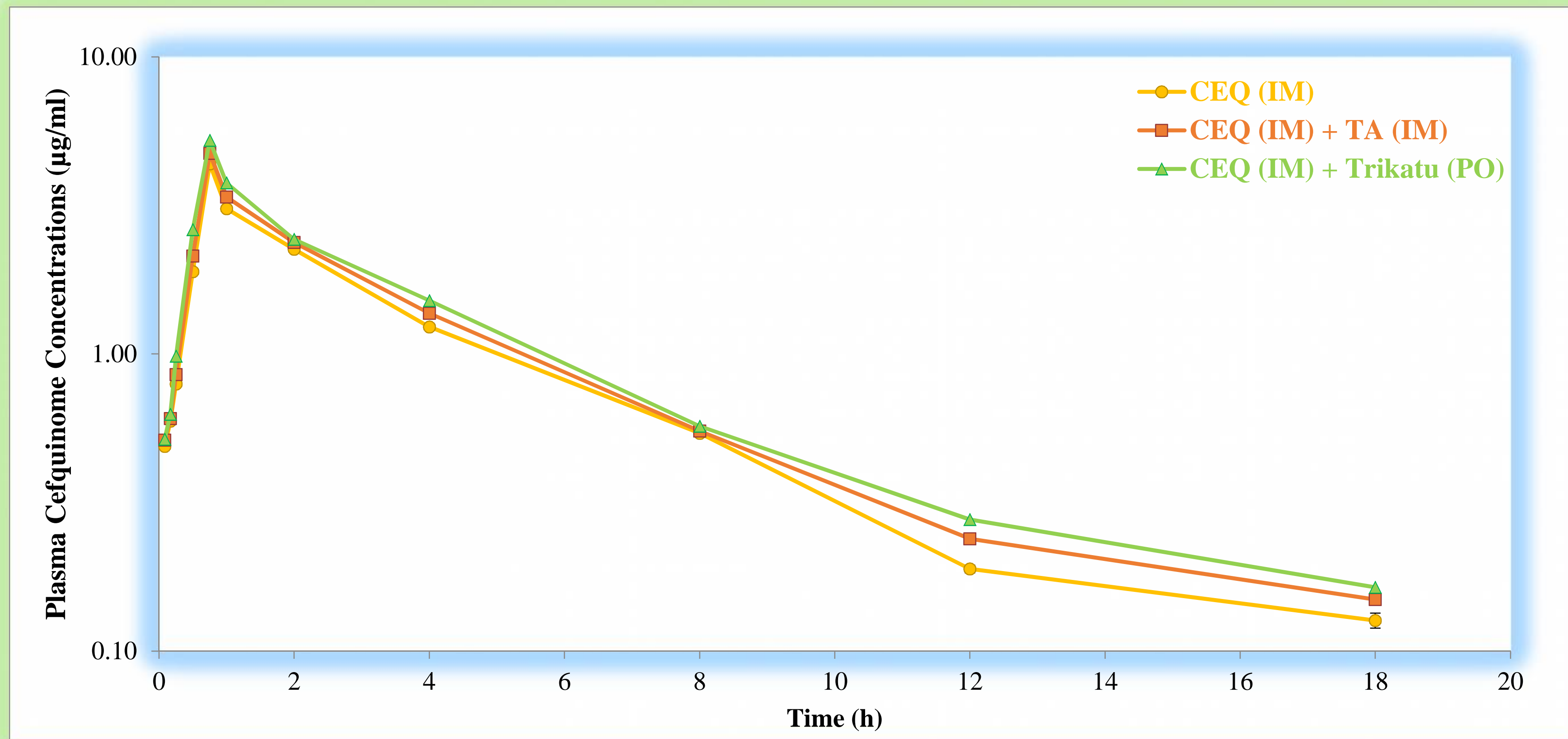

Figure 4.5: Semi logarithmic plot of cefquinome concentration in plasma versus time following intramuscular administration of cefquinome (2 mg/kg), tolfenamic acid treated (2 mg/kg) and trikatu pretreated sheep. Each point represents mean ± SE of six animals.

Table 2.3: Pharmacokinetic parameters of cefquinome in various species of animals.

Animal Species	**Dose (mg/kg)**	**Route**	**Pharmacokinetic Parameters**									**References**
			$t_{1/2s}$ (h)	**$Vd_{(area)}$ (L/kg)**	**$Vd_{(ss)}$ (L/kg)**	**$Cl_{(B)}$ (L/h/kg)**	**AUC (~g.h/mL)**	**C_{max} (~g/mL)**	**T_{max} (h)**	**MRT (h)**	**F (%)**	
Buffalo calves	2	IV	3.56 ± 0.05	0.31 ± 0.008	0.26 ± 0.006	60.9 ± 1.09	32.9 ± 0.56	-	-	4.24 ± 0.09	-	Dinakaran *et al.* (2013)
Goats	2	IV	5.76 ± 0.19	0.51 ± 0.05	0.37 ± 0.02	0.06 ± 0.004	33.83 ± 2.53	-	-	6.09 ± 0.11	-	Dumka *et al.* (2013)
		IM	5.86 ± 0.29	-	-	-	19.82 ± 2.07	4.84 ± 0.23	1.50 ± 0.00	8.08 ± 0.50	57.39 ± 3.40	
Chickens	2	IV	1.29 ± 0.10	-	0.49 ± 0.05	0.35 ± 0.04	5.33 ± 0.55	-	-	-	-	Xie *et al.* (2013)
		IM	1.35 ± 0.20	-	-	-	5.13 ± 1.06	3.04 ± 0.71	0.25± 0.06		95.81 ± 5.81	
Piglets	2	IM (Neck)	1.57 ± 0.23	-	-	-	11.44 ± 1.51	4.62 ± 0.31	0.38 ± 0.14	2.07 ± 0.29	103.0 ± 13.01	Song *et al.* (2013)
		IM (Thigh)	1.56 ± 0.02	-	-	-	9.29 ± 1.18	4.39 ± 0.53	0.42 ± 0.13	2.05 ± 0.21	97.56 ± 6.14	
Sheep	2	IV	0.78 ± 0.19	-	0.36 ± 0.06	0.34 ± 0.03	5.83 ± 0.45	-	-	3.74 ± 1.21	-	Uney *et al.* (2011)
		IM	1.88 ± 0.40	-	-	-	5.19 ± 0.25	2.60 ± 0.14	0.50	5.24 ± 1.12	98.31 ± 6.06	

Sheep	1 (One month)	IM	4.79 ± 0.36	-	-	-	9.70 ± 0.827	0.732 ± 0.04	3.81 ± 0.04	8.15 ± 0.98	-	Tohamy (2011)
	1 (Six months)		4.47 ± 0.24	-	-	-	13.17 ± 1.08	1.145 ± 0.074	3.02 ± 0.173	6.93 ± 0.40	-	
	1 (One year)		3.91 ± 0.19	-	-	-	12.18 ± 1.103	1.205 ± 0.078	2.17 ± 0.05	5.30 ± 0.18	-	
	10 (One month)	IM	3.33 ± 0.22	-	-	-	36.25 ± 3.22	3.52 ± 0.17	3.78 ± 0.226	7.27 ± 0.44	-	
	10 (Six months)		2.47 ± 0.31	-	-	-	42.21 ± 3.80	5.088 ± 0.333	2.82 ± 0.087	4.75 ± 0.47	-	
	10 (One year)		2.41 ± 0.19	-	-	-	40.40 ± 3.18	4.57 ± 0.338	3.09 ± 0.351	5.13 ± 0.370	-	
Ducks	5	IV	1.57 ± 0.06	-	0.41 ± 0.04	0.22 ± 0.02	25.12 ± 2.31	-	-	-	-	Yuan *et al.* (2011)
		IM	1.79 ± 0.13	-	-	-	23.78 ± 3.87	9.38 ± 1.61	0.38 ± 0.06	-	93.28 ± 13.89	
Rabbit	2	IV	0.93 ± 0.14	-	0.21 ± 0.03	0.18 ± 0.05	-	-	-	-	-	Hwang *et al.* (2011)
		IM	1.04 ± 0.22	-	-	-	-	8.87 ± 2.07	0.25 ± 0.12	-	95.23 ± 9.84	
Horses	1	IV	2.77 ± 1.03	-	0.21 ± 0.04	0.12 ± 0.02	7.66 ± 1.53	-	-	-	-	Winther *et al.* (2011)
Camels	1	IM	10.24	-	-	-	20.37	1.23	4.25 ± 0.10	16.74	-	Al-Taher (2010)

Pigs	1	IV	1.34	0.24	-	0.26	3.97	-	-	-	-	Yang *et al.* (2009)
		IM	-	-	-	0.25	4.12	1.80	-	-	102.37	
Piglets	2	IV	1.85 ± 1.11	-	0.46 ± 0.10	0.26 ± 0.08	8.07 ± 1.91	-	-	-	-	Li *et al,* (2008)
		IM	4.36 ± 2.35	-	-	-	-	4.01 ± 0.57	-	-	95.13 ± 9.93	
Pigs	2	IV	2.32 ± 0.47	-	-	0.12 ± 0.03	18.35 ± 5.32	-	-	-	-	Lu *et al.* (2007)
		IM	4.92 ± 1.14	-	-	-	17.22 ± 4.11	3.36 ± 0.54	0.83 ± 0.28	-	85.13 ± 9.93	
Buffalo Calves	1	IM	12.86 ± 1.60	-	-	-	17.76 ± 1.97	0.65 ± 0.01	7.53 ± 0.30	21.96 ± 1.01	-	Tohamy *et al.* (2006)
Cattle Calves			13.46 ± 1.80	-	-	-	18.67 ± 1.07	0.76 ± 0.09	4.60 ± 0.28	20.8 ± 1.61	-	
Cows			7.10 ± 0.73	-	-	-	8.92 ± 0.53	0.58 ± 0.08	4.07 ± 0.19	11.8 ± 1.07	-	
Goats			8.68 ± 0.91	-	-	-	14.36 ± 1.2	0.60 ± 0.07	9.23 ± 0.76	18.9 ± 1.0	-	
Dogs	5	IV	0.85 ± 0.10	-	0.20 ± 0.06	0.19 ± 0.04	-	-	-	-	-	Limbert *et al.* (1991)
Calves	10	IV	1.33 ± 0.41	-	0.23 ± 0.13	0.13 ± 0.06	-		-	-	-	

Table 2.4. Therapeutic Efficacy of cefquinome and other fourth generation cephalosporins.

Indications	Treatment	Remarks	References
Comparison of the clinical efficacy of cefquinome with penicillin G and gentamicin in equine	Cefquinome, Gentamicin, Penicillin G	❖ Cefquinome had greater efficacy than the combination of penicillin and gentamicin.	Widmer *et al.* (2009)
Clinical Mastitis in Cows and buffaloes	Cefquinome	❖ Cefquinome may be used as broad spectrum anti-infective agent for providing adequate clinical and bacteriological cure rates in clinical mastitis.	Palanivel *et al.* (2005)
Bovine endometritis	Cefquinome	❖ Cefquinome may be useful in the control of bovine metritis, especially in cases of reduced efficacy of older generation antimicrobial agents	Amiridis *et al.* (2003)
Acute Meningitis in Weaned Piglets	Cefquinome	❖ Cefquinome is effective for treatment of bacterial meningitis in piglets.	Thomas *et al.* (2002)
Acute Respiratory Disease	Cefquinome	❖ Cefquinome had a 98.1% clinical efficacy against acute respiratory disease in Holstein Steers as comparable to that of ceftiofur.	Funaki *et al.* (2001)

Coliform Mastitis	Cefquinome	❖ Cefquinome significantly improved clinical recovery and return to milk production. ❖ The bacteriological cure rates were considerably and significantly higher. ❖ Cefquinome is effective in the treatment of clinical coli form mastitis in dairy cows.	Shpigel *et al.* (1997)
Puerperal septicemia and toxemia in gilts	Cefquinome, Amoxicillin	❖ Cefquinome at doses of 2 mg/kg and 4 mg/kg body weight is clearly more effective than the control drug Amoxicillin in therapy of puerperal septicaemia and toxaemia.	Heinritzi and Hagn, (1999)
Pneumonia (late onset)	Cefpirome and cefepime	❖ Could be used as monotherapy because of its high potency against Gram-positive pathogens (*S. pneumonia* and *S. aureus*) and Gram-negative bacilli.	Jones *et al.* (1991) Spencer *et al.* (1997) Francioli *et al.* (1997)
Bacteremia / septicaemia	Ceftazidime and cefpirome	❖ Fourth generation is active against Gram positive cocci and *Enterobacteriacae* and stable to AmpC -lactamases and hence considered as first line of therapy ❖ Cefpirome (2 g, b.i.d) achieved satisfactory response in 97% of patients compared to 90% with ceftazidime	Norrby, (1993)

Febrile / neutropenia	Cefpirome, cefepime and ceftazidime	❖ Fourth generation cephalosporin are more active *in vitro* and *in vivo* and possesses enhanced activity against Gram positive bacteria and Enterobacteriaceae. Accordingly it may replace ceftazidime for empiric therapy in selected patients. ❖ Clinical response is reported as same (74 %) for both but bacteriological cure rate higher for Cefpirome (89%) to that of ceftazidime (74%)	Reiffers *et al.* (1992)
Bacterial Meningitis	Cefpirome and Cefepime	❖ Excellent activity against *Neisseria meningitides, H. influenza* and *S.pneumonia, S. aureus* for both	Linares *et al.* (1992) Fremaux *et al.* (1994) Barry *et al.* (1995)
Urinary tract, lower respiratory tract and wound infection	Cefpirome	❖ It had broad spectrum of activity against *Staphylococcus* spp, enterococci spp., *Enterobacter* spp., and *Pseudomonas* spp. Which are frequently resistant to third generation cephalosporins.	Schafer *et al.*(1992)
Experimental endocarditis	Cefepime , cefpirome	❖ Same efficacy reported for both	Lamb *et al.* (1993)
Gynaecological Infection	Cefpirome, cefmetazole	❖ Same efficacy reported for both	Itamochi *et al.* (2011)

Experimental bacteremia and meningitis in neonatal rats	Cefepime, Cefotaxime, Penicillin G	❖ Same efficacy to that of cefotaxime for *Escherichia coli* and penicillin G for group B *Streptococci spp.*	Kim and Bayer, (1985)
Complicated urinary tract, lower respiratory tract, skin and soft-tissue infections, and surgical infections	Cefepime, Cefpirome	❖ Cure rates of about 90% have been reported for both drugs.	Giamarellou (1996)
Experimental meningitis in neonatal rats	Cefepime, Ceftazidime, Cefotaxime, Moxalactam	❖ Highly efficacious for *Streptococcus agalactiae, Streptococcus pneumonia* and *Escherichia coli.* ❖ Same efficacious as cefotaxime against *Staphylococcus aureus*; as ceftazidime against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*;as moxalactam and cefotaxime against *Haemophilus influenza.*	Tsai *et al.*(1990)
Nosocomial lower respiratory tract infections in humans	Cefepime, Cefotaxime	❖ Cure rates for cefepime and cefotaxime are 73% and 56%, respectively. ❖ Bacteriological responses were 89% and 73% eradication from cefepime and cefotaxime treated groups, respectively.	Barckow and Schwigon (1993)
Nosocomial pneumonia in humans	Cefepime	❖ Cure rates for cefepime was 81%.	Jauregui *et al.* (1993)
Community-acquired bronchitis and pneumonia in humans	Cefepime, Ceftazidime	❖ Clinical response in patients treated with cefepime and ceftazidime were 87% and 86%, respectively; bacteriological responses were similar (95%).	Leophonte *et al.* (1993)

Table 2.5: Pharmacokinetic parameters of antibiotics with trikatu in animals.

Animal	Drug	Pharmacokinetic Parameters						References
		$t_{1/2s}$ (h)	Cl (B) (L/h/kg)	AUC (~g.h/mL)	C_{max} (µg/mL)	T_{max} (h)	MRT (h)	
Rats	Amoxycillin (100 mg/kg) PO	1.18 ± 0.48	-	10.73 ± 0.54	4.2 ± 0.28	1.5 ± 0.35	-	Hiwale *et. al.*, (2002)
	Amoxycillin (100 mg/kg) + Piperine (10mg/kg) PO	1.57 ± 0.39	-	17.80 ± 0.41	8.0 ± 0.34	1.0 ± 0.22	-	
	Amoxycillin (100 mg/kg) +Piperine (20 mg/kg) PO	1.63 ± 0.67	-	22.18 ± 0.49	9.4 ± 0.27	1.1 ± 0.3	-	
	Cefotaxime (10 mg/kg) IP	1.08 ± 0.57	-	32.64 ± 0.34	20.5 ± 0.3	0.5 ± 0.28	-	
	Cefotaxime (10 mg/kg) IP + Piperine (10 mg/kg) PO	1.56 ± 0.42	-	55.85 ± 0.45	31.0 ± 0.32	0.55 ± 0.35	-	
	Cefotaxime (10 mg/kg) Ip +Piperine (20 mg/kg) PO	1.78 ± 0.81	-	71.09 ± 0.42	35.13 ± 0.25	0.6 ± 0.31	-	
	Cefadroxil (100 mg/kg) PO	2.97 ± 0.75	-	152.92 ± 1.4	45.25 ± 1.09	1 ± 0.13	-	
	Cefadroxil (100 mg/kg) PO +Piperine (10 mg/kg) PO	2.85 ± 0.65	-	142.34 ± 1.5	45.5 ± 0.78	1 ± 0.15	-	
	Cefadroxil (100 mg/kg) PO +Piperine (20 mg/kg) PO	2.95 ± 0.85	-	152.97 ± 1.55	43.0 ± 0.93	1.0 ± 0.1	-	
Poultry	Oxytetracycline (10 mg/kg) PO	4.93 ± .422	184.63 ± 7.7	5.05 ± 0.68	-	-	7.98 ± 0.74	Singh *et. al.*, (2005)
	Oxytetracycline (10 mg/kg) + piperine (15 mg /kg) PO	6.37 ± .438	145.97 ± 14.42	6.41 ± 0.371	-	-	9.77 ± 0.64	

Animals	Drug	Pharmacokinetic Parameters						References
		$t_{1/2S}$ (h)	Cl (B) (L/h/kg)	AUC (~g.h/mL)	C_{max} (µg/mL)	T_{max} (h)	MRT (h)	
Goat	Pefloxacin	2.5 ± 0.12	0.29 ± 0.2	27.10 ± 0.38	-	-	4.47 ± 0.16	Dama *et al*. (2008)
	Pefloxacin + Trikatu (Pulverized powder)	3.30 ± 0.19	0.29 ± 0.2	30.85 ± 1.39	-	-	5.27 ± 0.27	
Cow calves	Enrofloxacin (5 mg/kg) PO	3.59 ± 0.2	0.48 ± 0.4	10.7 ± 0.97	-	-	9.78 ± 0.30	Sharma and Vaeshney (2009)
	Enrofloxacin (5 mg/kg) + Trikatu	-	-	15.88 ± 0.42	-	-	-	
Rabbits	Ampicillin (150 mg/kg) PO	1.3 ± 0.46	-	103.7 ± 0.52	44.6 ± 0.27	1±0.31	-	Janakiraman and Manavalan, (2008[a])
	Ampicillin (150 mg/kg) +Piperine (20 mg/kg) PO	1.9 ± 0.57	-	350.48 ± 0.47	251.2 ± 0.28	1.1±0.3	-	
	Norfloxacin (150 mg/kg) PO	1.75 ± 0.38	-	63.97 ± 0.51	11 ± 0.26	3.1±0.32	-	
	Norfloxacin (150 mg/kg) + Piperine (20 mg/kg) PO	2.97 ± 0.38	-	111.69 ± 0.54	16.1 ± 0.27	3.2±0.31	-	

Animal	Drug	Pharmacokinetic Parameters						References
		$t_{1/2s}$ (h)	Cl (B) (L/h/kg)	AUC (~g.h/mL)	C_{max} (µg/mL)	T_{max} (h)	MRT (h)	
Rabbits	Ampicillin	1.3 ± 0.46	-	103.71 ± 0.52	44. 67 ± 0.27	1.0 ± 0.31	-	Janakiraman and Manavalan, (2008[b])
	Ampicillin + *Piper nigrum* ME	1.6 ± 0.22	-	137.73 ± 0.28	74.98 ± 0.37	1.5 ± 0.25	-	
	Ampicillin + *Piper longum* ME	1.4 ± 0.23	-	118.48 ± 0.24	59.56 ± 0.38	1.0 ± 0.34	-	
	Ampicillin + *Zingiber officinale* ME	3.0 ± 0.28	-	126.72 ± 0.25	59.56 ± 0.38	1.0 ± 0.34	-	
	Ampicillin + Trikatu (ME combination)	1.4 ± 0.23	-	251.57 ± 0.26	149. 62 ± 0.25	1.0 ± 0.25	-	
	Ciprofloxacin (80 mg/kg) PO	3.61 ± 0.05	-	94.08 ± 1.7	13.42 ± 0.34	2.06 ± 0.16	-	Bhise and Pore, 2002
	Ciprofloxacin (80 mg/kg) + Piperine (10 mg/kg) PO	4.00 ± 0.042	-	160.24 ± 1.07	22.60 ± 0.25	1.55 ± 0.02	-	

Animals	Drug	Pharmacokinetic Parameters						References
		$t_{1/2s}$ (h)	Cl (B) (L/h/kg)	AUC (~g.h/mL)	C_{max} (µg/mL)	T_{max} (h)	MRT (h)	
Poultry	Gatifloxacin (10 mg/kg)PO	3.74 ± 0.073	0.66 ± 0.009	15.25 ± 0.21	1.74 ± 0.02	-	7.10 ± 0.07	Dama *et al.* (2008)
	Gatifloxacin (10 mg/kg)+ Piperine (15 mg/kg)PO	4.03 ± 0.097	0.57 ± 0.006	17.54 ± 0.20	2.14 ± 0.01	-	7.34 ± 0.09	
Sheep	Gatifloxacin (10 mg/kg) PO	4.05 ± 0.19	0.81 ± 0.17	12.44 ± 0.26	1.62 ± 0.02	-	6.95 ± 0.16	Walunj (2008)
	Gatifloxacin + Trikatu	4.39 ± 0.16	0.66 ± 0.01	15.34 ± 0.31	1.80 ± 0.03	-	7.74 ± 0.19	
Goats	Levofloxacin (4 mg/kg) PO	0.95 ± 0.065	1.06 ± 0.059	2.86 ± 0.139	0.60 ± 0.031	-	4.05 ± 0.094	Patel (2012)
	Levofloxacin (4 mg/kg) PO + Trikatu pretreated goat	2.07 ± 0.15	1.02 ± 0.04	4.15 ± 0.10	0.74 ± 0.03	-	4.95 ± 0.15	
	Levofloxacin (4 mg/kg) PO + *Piper longum* pretreated goat	1.98 ± 0.08	1.02 ± 0.04	3.63 ± 0.14	0.64 ± 0.02	-	5.06 ± 0.09	
	Levofloxacin (4 mg/kg) PO + *Piper nigrum* pretreated goat	2.04 ± 0.17	1.01 ± 0.04	3.83 ± 0.11	0.80 ± 0.02	-	5.22 ± 0.14	
	Levofloxacin (4 mg/kg) PO + *Zingiber officinale* pretreated goat	0.98 ± 0.07	1.08 ± 0.07	2.88 ± 0.11	0.59 ± 0.02	-	4.04 ± 0.10	