PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF CEFTIOFUR IN BUFFALO CALVES

Dissertation

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY
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VETERINARY PHARMACOLOGY AND TOXICOLOGY
(Minor Subject: Animal Biotechnology)

By

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CERTIFICATE – I

This is to certify that the dissertation entitled, **"PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF CEFTIOFUR IN BUFFALO CALVES"** submitted for the degree of **Ph.D.,** in the subject of **Veterinary Pharmacology and Toxicology** (Minor Subject: **Animal Biotechnology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Daundkar Prashant Sudamrao (L-2011-V-22-D)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that the dissertation entitled, **"PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF CEFTIOFUR IN BUFFALO CALVES"** submitted by **Daundkar Prashant Sudamrao (L-2011-V-22-D)**, to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of Ph.D., in the subject of **Veterinary Pharmacology and Toxicology** (Minor Subject: **Animal Biotechnology**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

Pharmacokinetic (PK) and pharmacodynamic (PD) parameters of ceftiofur sodium (2 $mg.kg^{-1}$ B.W.) were studied in healthy as well as experimentally induced febrile and hepatic dysfunctioned buffalo calves along with *in vitro* plasma protein binding. The drug followed three compartment open model after i.v. whereas two compartment open model after i.m. and s.c. administration. Plasma exposure was good irrespective of the route administered. Decreased ability of enzyme system to metabolize ceftiofur resulted in rapid elimination of ceftiofur after i.v. dosing as compared to i.m. and s.c. Bioavailability of drug was fair after i.m. and s.c. administration. Plasma concentrations of parent drug and its metabolite DFC were lower in febrile animals as compared to healthy ones. There was rapid distribution, reduced plasma exposure and faster elimination of the drug as observed by significantly increased body clearance and shortened elimination half life in febrile animals as compared to healthy ones. It was found that plasma concentrations were lower in hepatic dysfunctioned buffalo calves than healthy calves. There was significantly reduced persistence and plasma exposure along with better distribution of ceftiofur and DFC in these animals as compared to healthy ones. Increased body clearance of parent drug along with its metabolite further added the evidence of faster elimination from the body of diseased animals as compared to healthy ones. There was no significant change in the plasma protein binding of ceftiofur in healthy and disease states. The MICs of ceftiofur against *E. coli* and *S. aureus* were 0.20 and 0.35 $\mu g.ml^{-1}$ respectively. Upon PK-PD integration, it is recommended to repeat ceftiofur sodium administration at 24 h after i.v. and i.m. dosing & 36 h after s.c. dosing in healthy buffalo calves, whereas the dose should be repeated at 12h and 24h interval for the treatment of febrile and hepatic dysfunctioned animals.

Keywords: Ceftiofur, Pharmacokinetics, HPLC, Pharmacodynamics, Febrile, Hepatic dysfunction, PK-PD integration

Signature of Major Advisor

Signature of the student

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ABBREVIATIONS

µg	:	Microgram
ANOVA	:	Analysis of Variance
CCFA	:	Ceftiofur Crystalline Free Acid
DFC	:	Desfuroylceftiofur
DMSO	:	Dimethyl sulfoxide
Fig.	:	Figure
g	:	Gram
h	:	Hours
HPLC	:	High Performance Liquid Chromatography
viz.		For example
i.e.	:	That is
i.m.	:	Intramuscular
s.c.	:	Subcutaneous
In vitro	:	Outside the living body and in an artificial environment
In vivo	:	Inside the living body of an animal
i.v.	:	Intravenous
L	:	Litre
MBC	:	Minimum Bacterial Concentration
mg	:	Milli gram
MIC	:	Minimum Inhibitory Concentration
min	:	Minutes
ml	:	Milli litre
no.	:	Number
^{o}C	:	Degree Centigrade
ppm	:	Parts per million
s.c.	:	Subcutaneous
SEM	:	Standard error mean
SPSS	:	Statistical Package for the social science
B.W.	:	Body Weight
µg	:	Microgram
kg	:	kilogram

CHAPTER I

INTRODUCTION

The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus *Cephalosporium acremonium*. Cephalosporins have been classified according to their chronological sequence of development into five generations. Newer generation cephalosporins came in order to overcome resistant of β-lactamases producing bacteria and are characterized by extended spectrum of activities against microorganisms. Ceftiofur is a third generation cephalosporin antibiotic developed mainly for veterinary use. Besides its activity against gram negative bacteria, it also has good activity against anaerobic bacteria lacking in earlier generations.

Ceftiofur is most commonly used antibiotic in bovine treatment (Zwald *et al* 2004 and Sawant *et al* 2005). It has good spectrum of activity against Gram-positive as well as Gram-negative bacteria, including β lactamase producing strains because of presence of additional methoxyimino side-chain to the aminothiazole group of third-generation cephalosporin (Neu 1982, Yancey *et al* 1987 and Baere *et al* 2004). The spectrum of microorganisms against which ceftiofur is effective include *Pasteurella multocida, Mannheimia haemolytica, Haemophilus somnus, Arcanobacterium pyogenes, Fusobacterium necrophorum, Prevotella melaninogenica, Escherichia coli, Streptococci* and *Staphylococci* (FDA 1988, 1991, Berge *et al* 2006, Dore *et al* 2011, Liu *et al* 2010 and Witte *et al* 2011). It has been used exclusively for the treatment of some respiratory diseases in beef as well as dairy cattle, interdigital dermatitis in cattle and other animals (Salmon *et al* 1995). Treatment of mastitis and septic conditions in cattle also include exclusive use of this antimicrobial (Erskine *et al* 1995 and Stanek and Kofler 1998). The ceftiofur sodium is indicated mainly in shipping fever and acute bronchopneumonia which occurs after transport in beef cattle,

whereas in dairy cattle, enzootic calf pneumonia is the major indication (Sweeney and Smith 1990). Intrauterine infections causing metritis, retained fetal membranes can also be treated by ceftiofur (Scott *et al* 2005 and Drillich *et al* 2001, 2006 a,b). Some long acting preparations of ceftiofur have shown good results against acute bovine interdigital necrobacillosis after subcutaneous (s.c.) administration (Hibbard *et al* 2004).

It has been documented that patho-physiological changes following an infection can affect the pharmacokinetics of antimicrobials (Tohamy, 2008 and Baggot 1980). The pharmacokinetics of ceftiofur in pigs has been shown to be altered by a disease state (Tantituvanont *et al* 2009) and these changes may lead to either toxicity or loss of efficacy. In animals, the indiscriminate use of antibiotics is a global concern due to their impact on the environment, human health and the generation of antibiotic resistance (Vilos *et al* 2012). Therefore, for effective treatment and reduction of further complications, an appropriate change in dosage regimen is necessary (Tantituvanont *et al* 2009). Little experimental data is available about the pharmacokinetics of ceftiofur in infected/diseased buffalo calves. So, to evaluate antimicrobials in these animals is an important aspect of the pharmacokinetic study.

Fever is one of the most common manifestations of many infectious diseases and is reported to alter heart rate, renal blood flow, hepatic and splanchnic blood flow, diuresis, enzyme activities as well as endocrine function, thereby might alter the pharmacokinetics of drugs (Pawar and Sharma 2008). Bacterial endotoxin produces hepatic and renal dysfunction as well as hemodynamic depression during fever and there are significant alterations in hepatic function, resulting in changes in levels of various enzymes responsible for the metabolism of antimicrobials and thus biotransformation and elimination processes (Joshi and Sharma 2009 and Sharma and

Ul Haq 2012a). Febrile condition in addition, also produces changes in the permeability of biological membrane barrier and/or tissue and plasma pH and thus may modify the distribution pattern of drugs (Pawar and Sharma 2008). Patho-physiological changes occurring during fever is also known to modify the pharmacokinetics of antimicrobials in the animal body and ultimately the effectiveness of drug against bacteria (Rajput *et al* 2011). Therefore for a judicious therapy, it is essential to study the pharmacokinetics and formulate the dosage regimen of an antimicrobial in febrile state in which it is to be used clinically (Pawar and Sharma 2008), since, the dosage regimen achieved in healthy subjects cannot be generalized in clinical cases to treat diseased animals (Sharma and Ul Haq 2012).

Liver is the main site for biotransformation and elimination of majority of drugs. For antimicrobial agents that are extensively metabolized by liver, metabolism will be affected both by altered liver perfusion and protein binding (McKinnon and Davis 2004). Drug disposition may be altered through several pathophysiological mechanisms i.e. portal hypertension or decreased hepatobiliary clearance and modifications in the volume of distribution through reduced albumin synthesis. Therefore direct estimation of serum antibiotic concentrations could be the reasonable approach to manage antibiotic therapy (Westphal and Brogard 1993) in order to rationalize the dosage regimen in this kind of clinical conditions. Hepatic dysfunction might alter the disposition kinetics of antimicrobial drugs leading to a potential therapeutic problem (Sharma *et al* 2005). Although various tests are developed for prediction of liver impairment, none of these can determine drug dosing in patients with liver dysfunction (Amarapurkar 2011). Therefore, it is obligatory to conduct pharmacokinetic trials in such conditions for determination of dosage regimen. Since, pharmacokinetics of chemotherapeutic agents are markedly altered in disease

conditions, the dosage regimen obtained in healthy subjects cannot be always extrapolated in clinical cases to treat diseased animals (Joshi and Sharma 2009). So, a study of the effect of such conditions on the pharmacokinetics of an antibiotic is necessary.

The *in vivo* efficacy of cephalosporins directly correlates to the time length that the unbound concentration remains above minimum inhibitory concentration (MIC) as percent of dosing interval (Andes and Craig 2002). Ceftiofur has been reported to show time-dependent bacterial killing (Plumb 2008) therefore the length of duration for which concentration of ceftiofur and its metabolite remains above the MIC_{90} value is an important consideration (Craig 1995, Turnidge 1998 and McKellar *et al* 2004).

Plasma protein binding plays a vital role in distribution, elimination and therapeutic effectiveness of drugs. The biological half-life of ceftiofur increases because of protein binding through prevention of β lactam ring from breaking as well as decreased renal elimination (Hornish and Kotarski 2002). Protein bound fraction of the antibiotic does not have any antibacterial activity. It is only free drug that possesses this activity (Craig and Kunin 1976), so for evaluation of the potential efficacy of antibacterial, its plasma proteins binding should be known.

Rational antibiotic therapy requires dosage regimens to be optimized for its clinical efficacy as well as to minimize the selection and spread of resistant pathogens. Pharmacokinetic studies which provide a foundation for determination of satisfactory dosage regimen are relevant, when they are carried out in the species in which the antimicrobials are to be used clinically (Sharma and Ul Haq 2012a). The pharmacokinetics of ceftiofur has been conducted in cattle (Brown *et al* 1996, 2000, Halstead *et al* 1992, Okker *et al* 2002, Tohamy 2008 and Liu *et al* 2010), goat

(Courtin *et al* 1997 and Dore *et al* 2011) sheep (Craigmill *et al* 1997) and red deer (Drew *et al* 2004). The literature regarding the pharmacokinetics and plasma protein binding of this cephalosporin antibiotic in buffalo species - one of the greatest contributors in livestock sector in India is scarce. The studies in cattle and buffalo species have underlined the differences in the pharmacokinetics of cephalosporin antibiotic (Joshi and Sharma 2009). It is also recommended not to extrapolate the data of one species to other species of animal without conducting the detailed pharmacokinetic study (Sharma and Srivastava 2006). The literature regarding pharmacokinetics and pharmacodynamics of the ceftiofur in feverish and hepatic dysfunctioned buffalo calves is not available.

Keeping the above facts in view, the present study was conducted to investigate concentrations of ceftiofur after i.v., i.m. and s.c. administration in healthy buffalo calves and it's with those achieved after i.v. route in diseased buffalo calves:

1. Plasma levels, pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in healthy buffalo calves after single intravenous, intramuscular and subcutaneous administration.
2. Plasma levels, pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in febrile buffalo calves.
3. Plasma levels, pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in hepatic dysfunctioned buffalo calves.
4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ceftiofur against the microorganisms of veterinary importance.

CHAPTER II

REVIEW OF LITERATURE

The indiscriminate use of antibiotics while treating clinical bacterial infections have led to the development of resistance among microorganisms and further search for newer antibiotics in order to combat it. Discovery of cephalosporins was the notable turning point in chemotherapy of microbial diseases (Sharma, 2000). Cephalosporin class of β-lactam antibacterials are extensively used in veterinary medicine for the prevention and treatment of bacterial infections (Becker *et al* 2004). The basic structure of cephalosporin is 7-aminocephalosporanic acid nucleus which is made up of 6 membered dihydrothaizine ring fused with a four membered β-lactam ring (Figure 1 a). The additional dihydrothiazine ring confers steric advantages to the β-lactam ring in the terms of increased resistance to the action of β-lactamases (penicillinases), thus cephalosporins have an inherent broader spectrum of activity than that of penicillins (O' Callaghan, 1979, Maugh, 1981). Cephalosporins are often administered empirically and usually considered as first line of treatment for regular outpatient and in-hospital use. It has been employed commonly in veterinary practice for treatment of pneumonia, urinary tract infection, pyoderma, soft tissue infection, osteomyelitis as well as for pre and post surgical use.

In order to overcome the resistance of bacterial enzymes, the scientist have modified the structure of older antibiotics, so as to make them resistant to attack by these lactamases and also broaden their spectrum. That is the reason third generation cephalosporins were developed to fill lacunae of first and second generations hence referred as "extended spectrum" cephalosporins. Third generation cephalosporins has been used for resistant infections caused by *P. aeruginosa* or *E.coli* (Papich and Reviere 2009).

Ceftiofur – a third generation cephalosporin antibiotic has good activity against anaerobes including gram negative bacteria. The mechanism of action of ceftiofur like other cephalosporins is analogous to that of penicillin. Third generation cephalosporin bind to specific penicillin-binding proteins (PBP) that serve as drug receptors on bacteria, inhibit cell wall synthesis by blocking transpeptidation of peptidoglycan due to a structural similarity to acyl-D-alanyl-D-alanine (Figure 1b) results in a defective barrier and an osmotically unstable spheroplast and activate autolytic enzymes in the cell wall, that lead to bacterial cell death (Petri 2006).

General pharmacokinetic

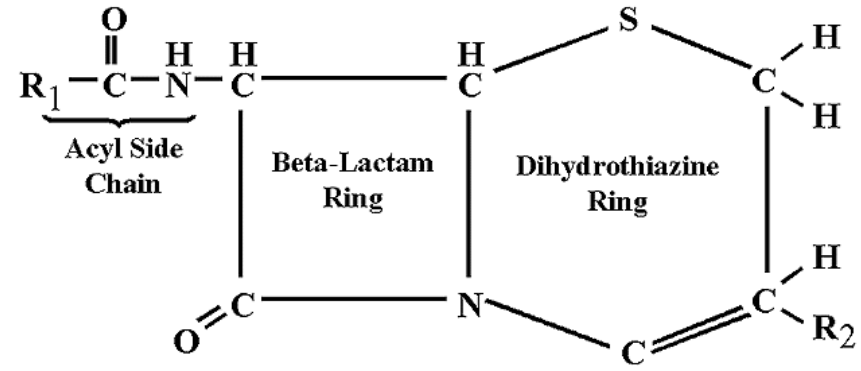
Pharmacokinetic studies provide a mathematical basis to measure the time course of drugs and their effects in the body. It also helps to understand absorption, distribution, metabolism and excretion of drugs (Toomula *et al* 2011).

The pharmacokinetic studies are based on mathematical modeling which are used to describe the changes in concentration of drug over a period of time. There are two approaches in this modeling i.e. compartmental and non-compartmental. In compartmental analysis, it is assumed that the rate of drug transfer between compartments and the rate of drug elimination from compartments follow first order or linear kinetics. These compartments are only mathematical entities and have no physiological or anatomical meaning (Riegelman *et al* 1968). In one compartment model, body is considered to be a single, kinetically homogenous unit. One compartment model is useful for the pharmacokinetics of drugs that can distribute rapidly throughout the body and so plasma concentration will be in equilibrium with other parts of the body. In this model, drugs mostly exhibit first order kinetics. Any change in blood drug concentration reflects the quantitative changes in tissue concentration. In this model, the plasma concentration time profile plotted on semi-

logarithmic scale yields a straight line and the equation to describe the drug decline is as follows:

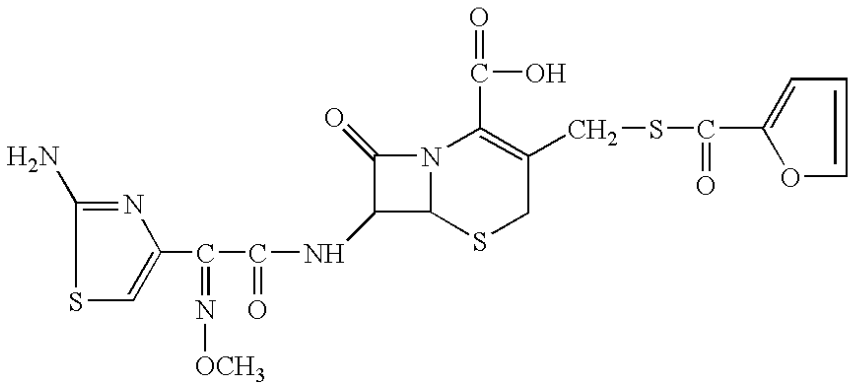
$$C_p = Be^{-\beta t}$$

Where, C_p denotes the plasma concentration of drug at time t, B is the Y- intercept of the regression line, β is the overall elimination rate constant and 'e' is the base of natural logarithm. This model has been found to adequately describe the kinetics of many cephalosporins including cefotaxime in buffalo calves (Sharma and Srivastava 2006), ceftriaxone in sheep (Tiwari *et al* 2010) and goats (Jimoh *et al* 2011).



General Structure of Cephalosporins

(a)



(b)

Figure: 1 a) General structure of cephalosporins b) Structure of ceftiofur

The two compartmental model assumes the existence of an instantaneous central compartment consisting of the blood and highly perfused organs such as liver,

kidney, lungs etc. and a peripheral compartment consisting of less perfused tissues such as muscle, skin etc. The addition of another compartment introduces a second exponential component in time course of plasma concentration plot, so that there are slow and fast decline phases in the graph. The transfer of drug from central to peripheral compartment (initial phase) is fast and is taken as distribution (α) phase wherein the drug molecules pass from blood to tissues hence lowering plasma concentration. The other phase is the slower one (β phase) which provides an estimation of rate of elimination. The equation to describe this model after i.v. and parenteral administration are as follows

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

$$C_P = Ae^{-\alpha t} + Be^{-\beta t} - A'e^{-Kat}$$

Where, A', A and B are zero time intercepts of initial absorption (parenteral route), distribution and elimination phase of log concentration time curve, respectively. The Ka, α and β are the rate constants for absorption (parenteral route), distribution and elimination, respectively.

The kinetic disposition of ceftiofur, has been reported to follow two compartmental open model in calves (Brown *et al* 1996), cattle (Whittem *et al* 1995), goats (Courtin *et al* 1997), sheep (Craigmill *et al* 1997) and cows (Tohamy 2008 and Liu *et al* 2010).

Three compartment open model is very much similar to two compartment open model except one more additional phase is observed. Some of drugs go at certain tissue site, where they are stored and released slowly for hours, days or even months. Owing to this the additional phase, slower distribution phase is obtained. For some drugs, the additional phase is obtained not because of slow elimination but due to their slow distribution in some tissues. The three and two compartmental open

model following i.v. and parenteral administration respectively is mathematically expressed as:

$$Cp = A_1e^{-\alpha 1t} + A_2\, e^{-\alpha 2t} + Be^{-\beta t}$$

$$C_P = Ae^{-\alpha t} + Be^{-\beta t} - A'e^{-Kat}$$

Where, A', A (A_1, A_2 for i.v. route) and B are zero time intercepts of initial absorption, distribution and elimination phase of log concentration time curve, respectively. The Ka, α (α_1, α_2 for i.v. route) and β are the absorption, distribution and elimination rate constants, respectively. Ceftriaxone in buffalo calves (Dardi *et al* 2004) and cows (Kumar *et al* 2010) are reported to follow three compartmental model.

Non-compartmental method does not require the assumption of a specific compartment division of body to study the disposition pattern of drug and/or its metabolite. The basis for non-compartmental analysis is the statistical moment theory. In pharmacokinetics, these moments are described by AUC (zero moment), AUMC (first moment) and VRT (second moment). Where AUC is the area under the plasma-concentration time curve, AUMC is the area under first moment curve and VRT is the variance of residence time (Rowland and Tozer 1989). These moments are calculated by using trapezoidal rule. The AUC and AUMC are invariably reported; the third moment is rarely used. The ratio of AUMC to AUC is a measure of its mean residence time (MRT). It provides a quantitative estimate of the persistence time of a drug in the body. In non-compartmental analysis, AUC is the basic parameter. The other important parameters like clearance and volume of distribution can be derived from it.

Blood/plasma levels and pharmacokinetics of ceftiofur in healthy conditions

At the basic level, pharmacokinetics is a tool to optimize the dosage schedule of drugs. Pharmacokinetic studies ensure safety and provide a suitable means to optimize the effective dosage regimen of drugs.

In a intravenous pharmacokinetic study of ceftiofur (2.2 mg ceftiofur free acid equivalents (CFAE/kg) in cows, $t_{1/2}\beta$, Cl_B, Vd_{SS} and AUC were 15.3±3.21 h, 0.0130 ± 0.0230 $ml.kg^{-1}.h^{-1}$, 0.298 ± 0.0350 $L.kg^{-1}$ and 163 ± 16.2 $\mu g.ml^{-1}.h^{-1}$ (Liu *et al* 2010).

Drillich *et al* (2006b) conducted an experiment on primiparous and multiparous cows having retained fetal membranes (RFM). They injected ceftiofur thrice at the dose rate of 1 $mg.kg^{-1}$ B.W. up to 72 h with 24 h interval between each dosing. They found that concentration of active metabolite, desfuroylceftiofur-acetamide (DFCA) was above minimum drug concentrations required to inhibit the growth of 90% of isolates of bacteria (*Escherichia coli*, *Fusobacterium necrophorum*, and *Arcanobacterium pyogenes*) causing infection (MIC_{90}) in serum, uterine tissues, cotyledons and lochia during the experimental period.

To study ceftiofur metabolites in plasma and uterine tissues, postpartum lactating Holstein-Friesian dairy cows were subcutaneously administered ceftiofur hydrochloride (1 $mg.kg^{-1}$ B.W.). C_{max} (2.85 ± 1.11 $\mu g.ml^{-1}$) was reached in plasma after 2 h and was decreased to 0.64 ± 0.14 $\mu g.ml^{-1}$ 24 h post injection. The concentration of active metabolite in lochial fluid, endometrium and caruncles were well above the reported MIC against common pathogens (*Escherichia coli*, *Fusobacterium necrophorum*, *Bacteroides spp*. and *Arcanobacterium pyogenes*) causing acute puerperal metritis (Okker *et al* 2002).

A pharmacokinetic study was conducted on twelve crossbred beef cattle following a single i.m. and s.c. administration of ceftiofur at the dose rate of 2.2 $mg.kg^{-1}$ B.W. (Brown *et al* 2000). They compared the bioequivalence of the sodium salt. The two criteria used were $AUC_{0\text{-}LOQ}$ i.e., area under the curve from time of injection to the limit of quantification and t > 0.2 i.e., time concentrations remained above 0.2 $\mu g.ml^{-1}$ for evaluation of bioequivalence. Similar C_{max} was observed by both the routes but T_{max} was less (0.67−2.0 h) when drug was administered through i.m. dosing than s.c. (0.67–3.0 h). The bioequivalence was found to be not differing much in either route. Depending upon their observations it was concluded that therapeutic efficacy and systemic safety for these two routes were similar.

To study the effect of age on the pharmacokinetic profile of the single i.m. and i.v. dose of ceftiofur (2.2 $mg.kg^{-1}$ B.W.), an experiment was conducted on day one old HF calves and one month old HF steers. Calves were given dose of ceftiofur at 7^{th} day, 1 and 3 month of the age whereas steers were injected at 6 and 9 months of age. Irrespective of the age, C_{max} were similar in both groups. Plasma concentrations were found to be greater than 0.150 $\mu g.ml^{-1}$ for 72 h in 7-day-old calves, but fallen below this limit within 48 h in steers irrespective of the route. Bioavailability after i.m. administration was 100%. The area under curve (AUC) in calves was significantly higher after i.v. injection than steers. The significant decrease in Vd_{ss} during initial 3 months was indicative of decrease extracellular fluid (ECF) volume after maturation. Significantly lower body clearance (Cl_B) during early age indicated underdeveloped metabolism and excretory mechanism for ceftiofur and DFC metabolites. It was suggested that dose of ceftiofur sodium 1.1-2.2 $mg.kg^{-1}$ for 5 successive days during bovine respiratory infection will provide plasma concentrations above the MIC for a longer period of time in neonatal calves than in steers. Peak concentrations of both

parent drug and its metabolite were similar in both neonates as well as mature animals (Brown *et al* 1996).

In a pharmacokinetic interaction study on non-pregnant, non-lactating dairy cattle, it was concluded that there was no need to change the dose as well as dosing interval of the ceftiofur (2 mg of ceftiofur sodium per kg body weight) when given i.v. along with acetyl salicylate (26 mg of aspirin/ kg body weight). In the same study, it was predicted that ceftiofur or its metabolites may interact with the organic anion transporter (OAT) namely probenecid (10 mg of probenecid/kg body weight) as shown by decrease in renal clearance (Cl) and mean residence time in peripheral (MRT_p) tissues (Whittem *et al* 1995). When ceftiofur was given as i.v. bolus (2 $mg.kg^{-1}$ B.W.), mean volume of distribution at steady-state (Vd_{ss}) was 0.2 ± 0.06 $L.kg^{-1}$, mean volume of distribution by the area method (Vd_{area}) was 0.38 ± 0.22 $L.kg^{-1}$, mean residence time (MRT) was 6.5 ± 1.8 h, MRT_p was 2.6 ± 1.0 h, total body clearance (Cl) was 0.032 ± 0.013 $L.kg^{-1}.h^{-1}$ and elimination rate constant (P) was 0.097 ± 0.044 h^{-1}.

In a dose dependent study, ceftiofur was evaluated for pharmacokinetic parameters in serum, tissue chamber fluid (TCF) and bronchial secretion (BS) after i.m. injection (2.2 and 4.4 $mg.kg^{-1}$ B.W.) at 24 h intervals for four successive days. The pharmacokinetic study was conducted after first as well as last dosing. After the first dosing, irrespective of the dose, peak serum concentrations (C_{max}) were observed following 2 h and the values were 8.8 $\mu g.ml^{-1}$ and 17.3 $\mu g.ml^{-1}$ for respective doses. At the end of final dosing there was approximately two fold increase in C_{max} (13.1 $\mu g.ml^{-1}$ and 24.1 $\mu g.ml^{-1}$) for respective doses. The peak concentration of antibiotic within TCF was 14% and 24% of the serum levels at respective sampling. Maximum levels reaching BS were 45% and 25%, respectively following single and multiple

dosing (2.2 and 4.4 $mg.kg^{-1}$ B.W.). It was concluded that the concentrations of ceftiofur after multiple dosing at 2.2 and 4.4 $mg.kg^{-1}$ B.W. was above MIC and can be used for treatment of acute respiratory diseases of calves (Halstead *et al* 1992).

An experiment was performed to know the difference of pharmacokinetics of crystalline free acid form of ceftiofur (6.6 $mg.kg^{-1}$ B.W.) in nonlactating and lactating adult female goats (Dore *et al* 2011). Significant difference in the pharmacokinetic parameters were not found between these two groups except for higher C_{max} (2.3± 1.1 $\mu g.ml^{-1}$) and less time for maximal observed serum concentration (T_{max} 26.7 ± 6.5 h) in nonlactating goats compared to lactating ones (C_{max} 1.5 ± 0.4 $\mu g.ml^{-1}$, T_{max} 46 ± 15.9 h).

In an experiment conducted on lactating goats given 1.1 mg ceftiofur free acid equivalents (CFAE)/kg and 2.2 mg CFAE/kg by i.v. and i.m. routes, the elimination half-lives ($t_{1/2}\beta$) after i.v. injection were 2.86 and 3.88 h for respective doses. There was significant decrease in $AUC_{0-\infty}$ and increase in the clearance (Cl) during lactation. The bioavailability of ceftiofur sodium was 100% after i.m. administration. The $t_{½}\beta$ was 2.72 and 2.60 h at two dose rates respectively in goats with dose dependent increase in $AUC_{0-\infty}$. Multiple dose study was performed on same goats after wash out period and concluded little accumulation along with increased concentration of drug with increased dose *via* i.m. route (Courtin *et al* 1997).

In a similar study on sheep administered ceftiofur sodium at 1.1 mg CFAE/kg B.W. or 2.2 CFAE/kg B.W. using two-route i.v. and i.m, the $t_{½}\beta$ after i.v. administration were 5.83 and 4.87 h and following i.m. route 6.48 and 7.65 h, respectively for these dose rates. Bioavailability of ceftiofur was 100% with dose dependent increase in AUC_{0-LOQ} when given intramuscularly. After 14 days of washout period, sheep were given ceftiofur daily for five days by i.m. route at the

dose rate of 1.1 and 2.2 mg CFAE/kg and they observed less drug accumulation as assessed by the observed C_{max} and the serum concentrations were dose dependent (Craigmill *et al* 1997).

Drew *et al* (2004) conducted a pharmacokinetic study on adult female red deer (*Cervus elaphus*). Ceftiofur sodium (250 mg) was administered intramuscularly as well as by ballistic implant. It was observed that pharmacokinetics of ceftiofur differed in both routes. There was a lag-time before detecting in plasma following ballistic implants in contrast to rapid absorption through i.m. route. The C_{max} was more in i.m. study than implants but no change in AUC was seen in both studies. The plasma concentration remained above MIC against the susceptible bacterial pathogens for 12 h on i.m. dosing but it was variable (4-24 h) after implantation. On the basis of their observations it was advocated that intramuscular route is better for antibiotic as efficacy after ballistic implantation remained questionable because of slow absorption as well as variable plasma concentration above MIC.

When ceftiofur was given at the dose of 1.1 $mg.kg^{-1}$ B.W. to asian elephants (*Elephas maximus*) through i.v. and i.m. routes, 19 % bioavailability was reported (Dumonceaux *et al* 2005). They recommended i.m. administration for the treatment of common pathogens in comparison to single i.v. injection.

Meyer and co-workers (2009) did a pharmacokinetic study of ceftiofur in foals at a dose of 5 $mg.kg^{-1}$ B.W. It was found that desfuroylceftiofur acetamide (DCA) had $t_{½}\beta$ of 7.8 ± 0.1 h, Cl_B 74.4 ± 8.4 $ml.h^{-1}.kg^{-1}$ and an apparent volume of distribution of 0.83 ± 0.09 $L.kg^{-1}$. They concluded that microbiologic assay estimation was insufficient for DCA activity. The MIC_{90} of ceftiofur was less than 0.5 $\mu g.ml^{-1}$ against *Escherichia coli*, *Pasteurella spp*., *Klebsiella spp*. and hemolytic *Streptococci*. The dose of 5 $mg.kg^{-1}$ b.i.d. for 5 consecutive days is sufficient to inhibit these bacteria.

Induction of experimental fever

Lipopolysaccharide (LPS) is a structural part of the outer cell wall of Gram-negative bacteria. It is normal inhabitant of gastrointestinal microflora in cattle without causing disease. However, even small amounts of LPS entry into the systemic circulation by any means produces severe clinical responses concomitant to systemic inflammation. LPS induced hyperthermia is mediated chiefly by the early production of prostaglandin E_2 within the brain and subsequent release of interleukin-1. These systemic effects after LPS challenge are observed at extremely low doses and differ significantly in potency and duration between individuals. This explains the need of repeated administration of LPS in some animals as differences in the ability of some individual to resist and tolerate an inflammatory insult (Jacobsen *et al* 2005).

The physiological effects of LPS challenge are based principally on activation and subsequent release of various molecular mediators *viz*. the cytokines, tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 by monocytes and macrophages. These inflammatory mediators initiate host inflammatory response and then production of hepatic acute phase proteins (APPs) which plays a key role in the host defence in bovines (Orro *et al* 2004). These interleukins and TNF also called as endogenous pyrogens are responsible for the fever following endotoxin challenge (Gordon *et al* 2005).

There are several ways to induce fever experimentally, but the best method is by intravenous injection of exogenous pyrogen, such as *E. coli* endotoxin (Musa *et al* 1972, Van Miert 1979). In several earlier studies, the dose of *E. coli* endotoxin, which can induce fever in buffalo calves (Joshi and Sharma 2009 and Sharma and Ul-Haq 2012), cross bred calves (Pawar and Sharma 2008 and Rajput *et al* 2008, 2011) after single/repeated intravenous administration at the dose rate of 1 $\mu g.kg^{-1}$ body weight

was administered intravenously, whereas, in sheep and goats 0.2 µg.kg^{-1} was given (Ranjan *et al* 2012 and Patel *et al* 2012a,b).

Pharmacokinetics of cephalosporins in febrile condition

An experiment was performed to know the pharmacokinetic profile of ceftiofur sodium (2.2 mg kg^{-1} B.W.) administered intravenously and intramuscularly along with dipyrone (NSAID) in healthy as well as feverish cows. After i.v. injection, Vd_{ss} and total body clearance (Cl_B) were significantly higher in feverish cows as compared to healthy ones. Following i.m. dose, the drug was absorbed at a faster rate in feverish than in healthy cows as shown by short absorption half-life ($t_{1/2}\alpha$). C_{max} in feverish cows was lower than healthy ones and was achieved (T_{max}) in less time than healthy ones. Less $t_{1/2}\alpha$ indicated rapid and fast absorption, greater Vd_{ss} and Cl_B correspond to the more drug penetration in body tissues and faster drug elimination from the body. Lower C_{max} may be attributed to rapid and fast absorption as well as distribution of the antibiotic to body tissues (Tohamy 2008).

The pharmacokinetic properties of ceftazidime were studied in experimentally induced febrile buffalo calves after a single i.v. administration at the dose rate of 10 mg.kg^{-1} B.W. (Sharma and Ul Haq 2012). They reported that the plasma levels of the drug in febrile animals were almost similar to healthy ones. The $t_{1/2}\beta$ (3.73 ± 0.42 h) of ceftazidime in febrile animals was parallel to that reported in healthy buffalo calves. The value of Vd_{ss} (0.2 ± 0.02 L.kg^{-1}) was consistent with that reported in healthy animals. Similarly, Cl_B (47.9 ± 4.57 ml.kg.h^{-1}) was also not significantly different as compared to healthy subjects. Based on their study they concluded that febrile conditions did not affect the pharmacokinetics of drug in buffalo calves. It might be because ceftazidime is not significantly metabolized, is excreted unchanged principally in urine by the glomerular filtration process and also poorly bound to plasma proteins.

The pharmacokinetics of cefepime after its single i.v. administration (10 $mg.kg^{-1}$ B.W.) was studied in experimentally induced fever in buffalo calves. The fever was induced by single/repeated i.v. injection of *E. coli* lipopolysaccaride (1 $\mu g.kg^{-1}$). The drug was estimated in plasma samples by microbiological assay using *E. coli* (MTCC 739) as a test organism. C_{max} of cefepime in febrile buffalo calves (40.8 ± 0.98 $\mu g.ml^{-1}$) was almost identical to healthy buffalo calves. Similarly, $t_{1/2}\beta$ in febrile animals (3.00 ± 0.18 h) was analogous to that reported in healthy animals. The value of Cl_B (98.8 ± 6.06 $ml.kg^{-1}.h^{-1}$) was almost identical to that of healthy buffalo calves. From the investigation they concluded that cefepime is not significantly metabolized in the liver, but excreted unchanged, mainly through urine by the glomerular filteration process and is poorly bound to plasma proteins. So, febrile conditions did not affected the pharmacokinetics of the antimicrobial. Authors recommended a dosage regimen of 7 $mg.kg^{-1}$ B.W. repeated at 12 h intervals in febrile buffalo calves to maintain a minimum therapeutic concentration of 1 $\mu g.ml^{-1}$. They also observed the alteration in the dosage regimen of the cefepime in febrile buffalo calves as compared to healthy caves where recommendation was 8.2 $mg.kg^{-1}$ B.W. at 8 h interval (Joshi and Sharma 2009)

Rajput *et al* (2008) investigated the influence of experimentally induced fever on disposition of cefpirome in buffalo calves. They observed plasma levels of cefpirome maintained above the therapeutic plasma concentration (MIC 0.39 $\mu g.ml^{-1}$) for lesser duration (12 h) after i.v. administration than healthy animals. The drug was distributed rapidly into various body fluids and tissue compartments than afebrile animals as indicated by higher value of distribution rate constant (13.9 ± 1.02 h^{-1}) and shorter $t_{1/2}\alpha$ (0.05 ± 0.003 h). The high value of K_{12}/K_{21} ratio (2.77 ± 0.20) than that in healthy subjects designated the rapid transfer of the antibacterial from central to

peripheral compartments in febrile buffalo calves. The value of AUC (35.1 ± 0.46 μg.ml^{-1}.h) displayed that adequate area of the body was exposed to cefpirome concentrations compared to healthy animals. The $t_{1/2}\beta$ (1.81 ± 0.009 h) and Cl_B (0.29 ± 0.004 L.kg^{-1}.h^{-1}) revealed rapid elimination and clearance of the drug from the body of febrile buffalo calves than healthy. Based on the study they recommended the dosage of 6 mg.kg^{-1} B.W. repeated 8 hourly in feverish buffalo calves quite different than healthy animals (8 mg.kg^{-1} B.W. repeated after 12 h).

Pharmacokinetics and dosage regimen of ceftriaxone was studied in *E. coli* lipopolysaccharide induced fever in buffalo calves. It was reported that C_{max} of ceftriaxone in febrile buffalo calves (79.4 ± 2.37 μg.ml^{-1}) was almost similar to healthy buffalo calves after i.v. administration. The values of Vd_{area} (1.21 ± 0.15 L.kg^{-1}) of ceftriaxone was lower in febrile animals when compared to healthy animals. The calculated values of AUC (25.2±1.97 μg.ml^{-1}.h^{-1}) in febrile buffalo calves were lower than the values reported in healthy animals. The value of Cl_B in febrile animals (0.41 ± 0.03 L.kg^{-1}.h^{-1}) was significantly higher as compared to the healthy animals (Dardi *et al* 2005).

Modification of the pharmacokinetics and dosage of cefuroxime (10 mg.kg^{-1} B.W.) by endotoxin-induced fever in buffalo calves has been reported (Chaudhary *et al* 1999). Significantly lesser initial plasma concentration of the drug was observed in febrile calves (66.4 ± 4.78 μg.ml^{-1}) than that in healthy one. The drug was detected for longer duration (9 h) in febrile animals as compared to healthy subject. Slightly prolonged $t_{1/2}\beta$ (2.28±0.28 h) and significantly increased MRT (2.54±0.22 h) values shown that the drug is excreted comparatively slowly in febrile animals than healthy. The values for distribution half-life ($t_{1/2}\alpha$), AUC, V_d and tissue/plasma (T/P) ratio were analogous in healthy and feverish animals. The study concluded that fever was

not able to alter the distribution of cefuroxime in buffalo calves. The calculated priming (10.8 ± 2.93 mg.kg^{-1} B.W.) and maintenance (10.1±2.99 mg.kg^{-1}B.W.) doses of cefuroxime for febrile buffalo calve were lesser than healthy calves.

The pharmacokinetics of cefepime was investigated in healthy and febrile cross-bred calves following single i.v. administration (10 mg.kg^{-1} B.W.). The fever was induced with *E. coli* lipopolysaccharide (1 µg.kg^{-1} i.v.). The drug concentration in plasma was detected by microbiological assay method using *E. coli* (MTCC 739) test organism. The $t_{1/2}\beta$ (1.62 ± 0.09 h) of cefepime was increased in febrile animals compared to healthy (1.26 ± 0.01 h). Drug distribution was altered by fever as shown by higher volume of distribution (0.27 ± 0.02 L.kg^{-1}) than normal calves (0.19 ± 0.01 L.kg^{-1}). Values of Cl_B in healthy and febrile animals were 104.4 ± 2.70 and 114.2 ± 1.20 ml.kg^{-1}.h^{-1}, respectively. The investigators recommended that a satisfactory dosage regimen of the antimicrobial in healthy and febrile cross-bred calves would be 15.5 mg.kg^{-1} B.W. and 8.2 mg.kg^{-1} B.W., respectively, to be repeated at 8 h intervals to maintain minimum therapeutic concentration of 1 µg.ml^{-1} (Pawar and Sharma 2008).

In a pharmacokinetic study, cefuroxime was administered intravenously (10 mg.kg^{-1} B.W.) in healthy and febrile cow calves. Plasma concentrations at all time points were significantly lower in febrile animals as compared to healthy, indicating more distribution of drug in febrile condition. The drug remained for longer period in plasma of febrile calves as indicated by highly significant greater values of $t_{½}$ β (2.92 h) and significantly lesser body clearance (153.0 + 14.4 ml.kg^{-1}.h^{-1}) than healthy calves (Chaudhary *et al* 2001).

After a pharmacokinetic study of cefepime (20 mg.kg^{-1} B.W.) following intramuscular administration in febrile goats, all pharmacokinetic parameter remained

non-significantly altered except elimination half-life (6.64 ± 0.33 h) and peak serum concentration (39.23 ± 1.11 μg.ml^{-1}). The $t_{1/2}\beta$ was significantly increased compared to non-febrile goats (Patel *et al* 2012).

The pharmacokinetics of cefepime (20 mg.kg^{-1} B.W.) was studied after i.m. administration in febrile sheep (Patel *et al* 2012). Absorption half-life $t_{1/2}k\alpha$ (0.24 ± 0.02 h), $AUC_{0-\infty}$ (221.67 ± 15.42 μg.h.ml^{-1}) and bioavailability (148.20 ± 11.64 %) were significantly increased whereas Vd_{area} (0.73 ± 0.04 L.kg^{-1}) and Cl_B (0.09 ± 0.01 L.h^{-1}.kg^{-1}) of cefepime were significantly decreased after intramuscular administration.

The effect of fever on the pharmacokinetics of ceftriaxone was studied after i.v. administration at the rate of 50 mg/kg B.W. in Chhotanagpuri sheep. The log plasma drug concentration versus time curve best fitted into two compartment open model. The C_{max} was 16.33 per cent less in febrile sheep than normal sheep. In feverish sheeps, $t_{1/2}\alpha$ (0.04 h) was shorter, whereas $t_{1/2}\beta$ (0.92 h) was longer as compared to normal animals ($t_{1/2}\alpha$ = 0.06 h, $t_{1/2}\beta$= 0.6 h) . The values of Vd_{area} (0.91 L.Kg^{-1}) and P: C ratio (4.55) were higher in LPS treated sheep than untreated animals (Vd_{area} = 0.58 L.Kg^{-1}, P/C=1.97). It was concluded that ceftriaxone has greater distribution in the peripheral compartment during pyrexia. High values of K_{12} indicated rapid drug distribution in various body fluids and compartments. The authors recommended dosage regimen of 15 mg.kg^{-1} B.W. at 5 h interval for ceftriaxone by intravenous route (Ranjan *et al* 2012).

Induction of hepatic dysfunction

The liver is a vital organ that regulate metabolism of xenobiotics including antimicrobials and is responsible for maintenance of body homeostasis. Paracetamol overdose has been reported to produce cellular and metabolic liver damage (Mayuren *et al* 2010 and Ben-Shachar *et al* 2012).

Paracetamol also referred by another name in United States as acetaminophen (APAP) gets metabolized by conjugation with sulfate and glucoronidate and are excreted in the urine as an inert conjugate. Depending on dose, a fraction of APAP is converted into N-acetyl-p-benzoquinone imine (NAPQI) - a highly reactive toxic intermediate by several P450 cytochromes (Fig. 2). Significant amounts of NAPQI are efficiently eliminated by conjugation with glutathione (GSH). However, after a large dose of APAP, there is saturation of sulfonation reaction and subsequently accumulated NAPQI depletes GSH in the liver. Unconjugated NAPQI binds to proteins and cellular structures and produces rapid cell death and necrosis that can lead to liver failure (Ben-Shachar *et al* 2012).

Kidney another crucial organ for metabolism and excretion of many xenobiotics including antibiotics is at risk of acetaminophen toxicity (Gulnaz *et al* 2010, Pakravan *et al* 2007 and Pathan *et al* 2013). It is the second most sensitive organ of acetaminophen toxicity and renal dysfunction occurs among patients with marked hepatic injury though, nephrotoxicity after acute overdose may even occur in absence of hepatotoxicity (Gulnaz *et al* 2010).

Much of the recent works for the production of experimentally induced hepatic dysfunction have been carried out by administration of high doses of paracetamol (Mayuren *et al* 2010 and Ben-Shachar *et al* 2012). In an experiment on rats, paracetamol was administered at the dose rate of 500 $mg.kg^{-1}$ body weight for 48 h to induce hepatic damage (Garba *et al* 2009) whereas Kumar *et al* (2009) induced hepatic damage in broiler chicken by i.m. administration of paracetamol at the dose rate of 250 $mg.kg^{-1}$ B.W. Bhaumic and Sharma (1993) also produced liver damage in sheep by i.v. injection of paracetamol at the dose rate of 400 $mg.kg^{-1}$ B.W. Pharmacokinetic alterations were studied in hepatic dysfunctioned buffalo calves,

wherein liver dysfunction was produced by i.v. injection of paracetamol 250 $mg.kg^{-1}$ at day 1, followed by 50 $mg.kg^{-1}$ each on day 3 and day 5 (Saini 1998, Sharma *et al* 2005 and Sharma and Ul Haq 2012b).

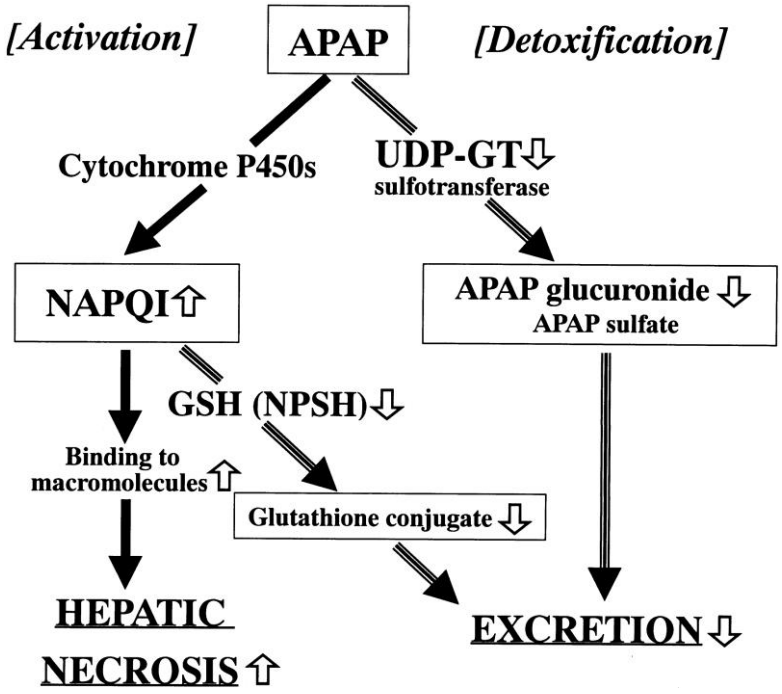


Figure 2: Hepatotoxic effect of Acetaminophen (APAP) (Enomoto *et al* 2001)

Pharmacokinetic of cephalosporins in hepatic dysfunctioned animals

Pharmacokinetics of ceftazidime was conducted in water buffalo calves (*Bubalus bubalis*) with hepatic dysfunction (Sharma and Ul Haq 2012b). Plasma levels of the drug in animals with hepatic-dysfunction were lower than reported in healthy water buffalo calves. There was no significant alteration in the $t_{1/2}\beta$ (3.58 ± 0.19 h) and the value of Vd_{ss} (0.19 ± 0.01 $L.kg^{-1}$) compared to healthy animals. The higher value of Cl_B (58.2 ± 1.64 $ml.kg^{-1}.h^{-1}$) in animals with hepatic-dysfunction in comparison with healthy water buffalo calves may be due to consequence of disease specific changes in enzyme activity and/or drug transport within the liver. On the basis of urinary excretion, authors advocated ceftazidime use at proposed route and dose rate up to 32 h for urinary tract infection in buffalo calves caused by

microorganisms having susceptibility ≤ 10 μg.ml^{-1}.

In an investigation, pharmacokinetics of cefotaxime after its single i.v. administration (10 mg.kg^{-1}) was studied in hepatic-dysfunctioned buffalo calves. Hepatic-dysfunction was induced by i.m. administration of paracetamol (250 mg.kg^{-1} B.W. on day 1, followed by two subsequent doses of 50 mg.kg^{-1} B.W. on day 3 and 5). The results of the study revealed marked differences in kinetic behaviour of cefotaxime in these animals as compared to healthy buffalo calves as indicated by considerably lesser $t_{1/2}\beta$ (0.94 ± 0.02 h) and higher value of Cl_B (1.1 ± 0.06 L.kg^{-1}.h^{-1}) of cefotaxime than healthy buffalo calves. The increased hepatic clearance of the drug may be the consequence of disease specific changes in both enzyme activity and/or drug transport within the liver (Sharma *et al* 2005).

Disposition kinetics of cefuroxime was investigated in healthy and hepatopathic buffalo calves by Kumar (1999). The drug was administered at the dose rate of 10 mg.kg^{-1} B.W. intramuscularly. Elevated peak plasma concentration were observed in hepatopathic calves (26.0 ± 0.61 μg.ml^{-1}) as compared to healthy calves (23.0 ± 1.50 μg.ml^{-1}). But MIC was maintained upto 4-5 h after administration in both groups. The values of pharmacokinetic parameters were almost similar in hepatopathic as well as healthy buffalo calves.

The pharmacokinetics of ciprofloxacin and pefloxacin (single i.v. for both at the dose rate of 5mg.kg^{-1}) were studied in healthy and hepatic dysfunctioned buffalo calves. Hepatopathy was induced by i.m. injections of paracetamol (cumulative dose of 350 mg.kg^{-1} B.W. for the period of five days). At several time points, the plasma concentrations of pefloxacin in hepatopathic buffalo calves were lower than healthy subjects. The values of $t_{1/2}\alpha$, $t_{1/2}\beta$, Vd_{area} and Cl_B of pefloxacin were reported to be 0.033 ± 0.001 h, 1.03 ± 0.05 h, 1.13 ± 0.05 L.kg^{-1} and 0.76 ± 0.01 L.kg^{-1}.h^{-1},

respectively in healthy buffalo calves while 0.036 ± 0.003 h, 0.97±0.04 h, 1.12±0.04 $L.kg^{-1}$ and 0.796±0.008 $L.kg^{-1}.h^{-1}$, respectively in hepatopathic animals. The $t_{1/2}\alpha$,$t_{1/2}\beta$, Vd_{area} and Cl_B were 0.048 ± 0.002 h, 1.14 ± 0.06, 1.44 ± 0.07 $L.kg^{-1}$ and 0.882 ± 0.035 $L.kg^{-1}.h^{-1}$, respectively in healthy buffalo calves whereas 0.072 ± 0.007 h, 2.55 ± 0.38, 2.31 ± 0.28 $L.kg^{-1}$ and 0.640 ± 0.034 $L.kg^{-1}.h^{-1}$, respectively in hepatopathic animals for ciprofloxacin. It was concluded that, for pefloxacin, the distribution was decreased and elimination was increased, while for ciprofloxacin, the distribution was increased and elimination was decreased during hepatopathy (Saini 1998).

Disposition of ceftriaxone in hepatopathic goats after single-intramuscular dosing was studied by Sar *et al* (2013). Twelve healthy goats were grouped equally into two groups, first group served as control and hepatopathy was induced by carbon tetrachloride in the second group. A single intramuscular dose of ceftriaxone at the dose rate of 50 $mg.kg^{-1}$ B.W. was administered to each group. Disposition of ceftriaxone in plasma of healthy goats showed a distinct absorption-reabsorption phase which was totally absent in hepatopathic goats. In hepatopathic animals the disposition of ceftriaxone showed only absorption and distribution/elimination phase. The drug persisted in plasma for 6 h in hepatopathic animals, whereas up to 2 h in healthy goats signifying longer persistence of antibiotic in the former group. Ceftizoxime, the active metabolite of ceftriaxone was detected in urine of healthy goats but not in diseased animals suggesting impairment of metabolism of the parent drug during hepatopathy.

The pharmacokinetic profile of ceftriaxone was studied in female healthy goats, induced hepatopathic as well as nephropathic goats afer a single i.v. dose at 50 $mg.kg^{-1}$. The drug followed one-compartment open model type of kinetics in all the animals. Ceftriaxone persisted for 2 h in plasma of hepatopathic goats compared to 1

h of healthy goats after i.v. dosing. Significantly higher value of $t_{½}\beta$ (0.32 ± 0.008 h) was seen in hepatopathic goats compared to healthy goats (0.19 ± 0.002 h). The parent drug was detected up to 24 h in urine of hepatopathic goats but not in urine of healthy subjects. On the contrary, its metabolite ceftizoxime was present in urine of healthy goats but not in urine of diseased subjects. Ceftizoxime was quantified in an adequate amount in nephropathic goats from 8 h to 12 h post dosing. Elimination half-life of ceftriaxone was significantly longer in nephropathic goats (0.38 ± 0.01 h) compared to healthy goats (0.19 ± 0.002 h). Ceftriaxone, not the metabolite was detected at 24 h and 48 h after dosing in urine of nephropathic goats, while only ceftizoxime not parent drug was detected in urine of healthy goats (Mandal *et al* 2008).

Disposition kinetics of amoxicillin after single oral administration (40 $mg.kg^{-1}$ B.W.) was studied in healthy, hepatopathic and nephropathic broiler chickens (COBB 400) by Bhar *et al* 2010. Hepatopathy was induced by oral administration of paracetamol (500 $mg.kg^{-1}$ B.W.) while nephropathy was induced by intravenous administration of uranyl nitrate (2 $mg.kg^{-1}$ B.W.). At 1 h plasma concentrations of amoxicillin in healthy, hepatopathic and nephropathic birds were 41.90 ± 5.59, 9.93 ± 0.76 and 38.75 ± 6.08 $\mu g.ml^{-1}$, respectively; 15.34 ± 1.99, 18.57 ± 1.66 and 67.40 ± 2.62 $\mu g.ml^{-1}$, respectively at 4 hr post dosing and 2.03 ± 0.28, 15.54 ± 0.82 $\mu g.ml^{-1}$ and 30.63 ± 1.58 $\mu g.ml^{-1}$, respectively at 24 h. C_{max} was observed at 1 h in healthy birds (41.90 ± 5.59 $\mu g.ml^{-1}$), at 8 h in hepatopathic birds (23.51 ± 1.64 $\mu g.ml^{-1}$) and at 4 h in nephropathic birds (67.40 ± 2.62 $\mu g.ml^{-1}$) oral administration of the antimicrobial. The drug was not detected in plasma beyond 24 h in healthy whereas 72 h in diseased birds. At most of the time points, the concentration of amoxicillin was significantly ($P < 0.01$) higher in hepatopathic and nephropathic birds compared

to healthy ones. Significantly higher absorption half life ($t_{1/2}K_{abs}$) in both hepatopathic (3.03 ± 0.15 h) and nephropathic (0.91± 0.21) birds compared to healthy (0.21 ± 0.01 h) birds suggested poor absorption of amoxicillin in these conditions. The elimination half life ($t_{1/2}K$) prolonged significantly in hepatopatic (32.30 ± 1.35 h) and nephropatic (19.62 ± 0.33 h) birds as compared to healthy (6.04 ± 0.13 h). Similarly, AUC and MRT values increased significantly in hepatopathic (935.98 ± 66.62 µg.hr.ml^{-1}, 47.54 ± 1.64 h) and nephropathic (1878.71 ± 101.02 µg.h.ml^{-1}, 27.25 ± 0.18 h) chickens than healthy (235.93 ± 29.78 µg.h.ml^{-1}, 8.86 ± 0.19 h).

Protein binding studies

Protein binding for most cephalosporins is low in animals compared to human e.g. ceftriaxone and cefazolin has 19-25 % in dogs as compared to 85-95 % in human (Bhavsar and Thaker 2014). Plasma protein binding plays a vital role in distribution, elimination and therapeutic effectiveness of the drugs. Ceftiofur has been reported to show 65% protein binding among various species (Brown *et al* 1991). The binding of ceftiofur to serum proteins was reported to be 29.63% in healthy cow calves (Tohamy 2008) but the literature regarding the pharmacokinetics and plasma protein binding in buffalo species is not available. The author reported 46.6 % binding of ceftiofur to goat serum proteins (Singh 2014). Cefquinome bound to plasma proteins to the extent of 11.4 % in buffalo calves (Dinakaran *et al* 2014) whereas *in vitro* serum protein-binding was reported to be 15.65 % and 14.42 % in sheep and goats respectively (El-Hewaity *et al* 2014).

Pharmacodynamics of ceftiofur

In a pharmacodynamic study, ceftiofur and DFC were tested for *in vitro* activity against organisms of veterinary importance like *Actinobacillus pleuropneumoniae, Pasteurella spp., Haemophilus somnus, Salmonella spp.,*

Escherichia coli, *Staphylococci* and *Streptococci*. Both parent compound and metabolite were found to be equally efficacious against the gram-negative organisms with MICs within one serial dilution. But in case of *Staphylococci*, that was not the case since MIC_{90} were 1.0 and 4.0-8.0 $g.ml^{-1}$ for parent drug and its metabolite respectively differing 2-3 serial dilutions. Despite of having 5 serial dilutions higher MICs for DFC (MIC_{90} = 0.03 $g.ml^{-1}$) against *Streptococci* than parent drug (MIC_{90} = 0.001 $g.ml^{-1}$), this difference is not clinically important since both are highly active against these pathogens. Thus except for *Staphylococci* the parent compound and its metabolites were active against all other organisms tested (Salmon *et al* 1996).

It has been reported that *P. multocida* (41/98, 41.8%) and *Actinobacillus pleuropneumoniae* (38/98, 38.8%) are the chief pathogens causing respiratory diseases (Holko *et al* 2004). These pathogens are of economic significance since because of their considerable financial loss in the livestock sector through production of respiratory diseases (Hennig-Pauka *et al* 2006). Ceftiofur has greater *in vitro* activity against these bacterial pathogens and is used comprehensively for the treatment of respiratory diseases in cattle, small ruminants, horses and swine (Deshpande *et al* 2000, Hornish and Kotarski 2002, Jacobson *et al* 2006 and Frye and Fedorka-Cray 2007).

In a comparative study by Salmon and co-workers (1995), MICs of ceftiofur irrespective of the country were found to be same *i.e.* less than 2.0 $\mu g.ml^{-1}$. (*Actinobacillus pleuropneumoniae*, $\leq$0.03; *Escherichia coli*, 1.0; *Pasteurella multocida*, $\leq$0.03; *Salmonella choleraesuis*, 1.0; *Salmonella typhimurium*, 2.0; *Streptococcus suis*, 0.25; *β Hemolytic streptococci* $\leq$0.03). MIC_{90} of ceftiofur reported for *Pasteurella haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, *Actinobacillus pleuropneumonia* and *Klebsiella pneumoniae* was 0.25 $\mu g.ml^{-1}$

(Salmon *et al* 1996 and Deshpande *et al* 2000). *In vitro* studies stated MIC values of the antibiotic against *Pasteurella multocida* and *Mannheimia haemolytica* isolates were 0.03 and 0.0075 $\mu g.ml^{-1}$ respectively (Sweeney *et al* 2008). MIC range of the drug was 0.25-0.5 $\mu g.ml^{-1}$ for *Staphylococcus aureus* and *Streptococcus pneumoniae.* (Zhang *et al* 2008).

In addition, various studies by different groups of workers in cattle and goat, ceftiofur was found to be effective against the common respiratory pathogens like *Pasteurella multocida* and *Mannheimia haemolytica* with MIC value of 0.2 $\mu g.ml^{-1}$ (Berge *et al* 2006 and Dore *et al* 2011). Liu and co-workers (2010) determined MICs of ceftiofur sodium against *Escherichia coli, Streptococcus suis, Salmonella enteritidis* and *Staphylococcus aureus* were 1, 4, 1 and 2 $\mu g.ml^{-1}$ respectively. MIC_{90} value of ceftiofur against common pathogenic microorganisms such as *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, and *Prevotella melaninogenica* has been stated to be in the range 0.125–0.5 $\mu g.ml^{-1}$ (Witte *et al* 2011). MIC of ceftiofur against *Escherichia coli* (ATCC 25922) was 1 $\mu g.ml^{-1}$ (Vilos *et al* 2012).

PK-PD approach for dosage optimization

The pharmacokinetic (PK) studies illustrate the drug concentration of the antibiotic *in vivo* with respect to time *i.e.* in biological fluids (serum/plasma) or at the site of infection, whereas the pharmacodynamis (PD) studies provided the knowledge about characteristics of an antimicrobial i.e. sensitivity to target pathogen (MIC, MBC), inhibition of growth, rate and extent of antibacterial activity as well as post antibiotic effect (PAE). The main goal of PK-PD integration is to predict the outcome of antimicrobial therapy in clinical conditions. Thus, connecting the PK parameters with that of PD provides a rational basis for determination of optimal dosage regimen

in terms of the dose and the dosing interval (Toutain *et al* 2002, Levison, 2004 and Toutain and Lees 2004).

The time length that the concentration remains above minimum inhibitory concentration (MIC) as percent of dosing interval is a key paramter for *in vivo* efficacy of cephalosporins (Andes and Craig 2002). Significantly better clinical cure and bacteriological eradication was observed in serious bacterial infections after treatment with cephalosporins when the values of %T > MIC and AUIC were above 100 and 250 respectively than patients having AUIC < 250 (Peggy *et al* 2008).

Integration PK-PD parameters *viz*. maximum concentration of drug C_{max}/ MIC, the area under curve AUC/MIC and %T > MIC is important for dosage optimization of concentration dependent antibiotics so as to ensure bacteriological cure and minimize the risk of resistance (Aliabadi *et al* 2003). *In vitro* gram negative bactericidal action of cephalosporins have been reported to increase with increasing concentration above minimum bactericidal concentration therefore maintaining the levels above MIC will not be adequate since regrowth of bacteria can occur (Cholewka *et al* 1999).

CHAPTER III

MATERIALS AND METHODS

3.1 Animals

The experiment was conducted on six male buffalo calves of non-descript breed (weight 90-120 kgs, age 1 year) procured from market. The study was approved by Institutional Animal Ethics Committee (Order No. VMC/14/1046-73 dated 7.04.2014). The animals were acclimatized in the animal shed of department under ambient conditions. Animals were dewormed using endectoparasiticide ivermectin (Neomec^{R}) at the dose rate of 0.2 mg.kg^{-1} B.W. subcutaneously one month before start of experimentation and vaccinated against major diseases *viz*. Foot & Mouth disease, Haemorrhagic septicemia, Black Quarter as per the manufacturer (Punjab veterinary vaccine institute) protocol. Animals were given concentrate on body weight basis, green fodder of the season plus wheat straw and water were provided *ad libitum* throughout the experiment. All the animals were weighed empty stomach before the starting of the experiment for dose calculation on body weight basis.

3.2 Chemicals and reagents

Injectable form of ceftiofur sodium (ceftivet®) was purchased from Pfizer Animal Health India Ltd. Mumbai. Paracetamol (Paracetol-Vet®) was obtained from Cadila Health Care Ltd. Ahmedabad, India. *E. coli* endotoxins and Ceftiofur veternal standard were procured from Sigma Chemical Co., USA. Dithioerythritol (DTE), Iodoacetamide, Dimethyl Sulfoxide, Acetonitrile (ACN), methanol (MeOH), Trifluoroacetic acid (TFA) were purchased from Merck (Mumbai, India). Ultrapure analytical grade type I water for HPLC was produced by milli-Q water purification system (Millipore, Bedford, MA, USA). All reagents were of HPLC grade. Eosin Methylene Blue (EMB) Agar and Muellar Hinton Broth (MHB) were procured from Himedia Laboratories Pvt. Ltd. Mumbai. Mannitol salt agar was purchased from

Merck India Pvt. Ltd. Mumbai. Microwell plates (96 wells) with flat bottom of capacity 300 µl and 24 well tissue culture plates of capacity 3.5 ml were obtained from Orange Scientific, Belgium. Autopak kits for enzyme estimation were purchased from Bayer (Siemens Medical Solutions Diagnostics. Ltd. Baroda), Gamma-GT FS (Diasys Diagnostic Systems GmbH, Holzheim, Germany) and Erba diagnostic kits (Transasia Biomedicals Ltd. Mumbai).

3.3 Test organisms

Test Organism *Escherichia (E.) coli* (MTCC 739) and *Staphylococcus (S.) aureus* (MTCC 737) were obtained from Institute of Microbial Technology, Chandigarh.

3.4 Experimental design

The experiments were performed under following four major sections.

A. Pharmacokinetics of ceftiofur in healthy buffalo calves.
B. Pharmacokinetics of ceftiofur in experimentally-induced febrile buffalo calves.
C. Pharmacokinetics of ceftiofur in experimentally-induced hepatic dysfunctioned buffalo calves.
D. Pharmacodynamics of ceftiofur.

A. Pharmacokinetics of ceftiofur in healthy buffalo calves.

1. Plasma levels and pharmacokinetics after single intravenous administration.
2. Plasma levels and pharmacokinetics after single intramuscular administration.
3. Plasma levels and pharmacokinetics after single subcutaneous administration.
4. *In vitro* plasma protein binding of ceftiofur.

B. Pharmacokinetics of ceftiofur in febrile buffalo calves.

1. Plasma levels and pharmacokinetics after single intravenous administration.
2. *In vitro* plasma protein binding of ceftiofur.

C. Pharmacokinetics of ceftiofur in hepatic dysfunctioned buffalo calves.

1. Plasma levels and pharmacokinetics after single intravenous administration.
2. *In vitro* plasma protein binding of ceftiofur.

D. Pharmacodynamics of ceftiofur.

1. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ceftiofur against *E. coli* (reference strain MTCC 739).
2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ceftiofur against *S. aureus* (reference strain MTCC 737)

Plan and methodology

Grouping of animals

The animals were divided into different groups for various studies as per the experimental schedule shown in Table 1. The animals were reused after a gap of 3 weeks. Before repeating the drug in the same animals, the blood samples were collected and were made sure that there were no traces of drug.

Induction of fever

Fever was induced in buffalo calves following single/repeated intravenous administration of *E. coli* endotoxin at the dose rate of 1µg.kg^{-1} B.W. (Sharma and Ul-Haq 2012a and Joshi and Sharma 2009).

Induction of hepatic dysfunction

Liver dysfunction was induced in buffalo calves by intramuscular administration of paracetamol. The dosage schedule of paracetamol was 250 mg.kg^{-1} B.W. on day 1, followed by 2 subsequent doses of 50 mg.kg^{-1} B.W. on day 3 and day 5 (Sharma and Ul-Haq 2012b). The extent of liver dysfunction was assessed by daily

Table 1: Experimental schedule to study the pharmacokinetics, pharmaco-dynamics and *in vitro* plasma protein binding of ceftiofur in healthy and experimentally induced diseased buffalo calves

Group	Animal No.	Drug	Dose	Route	Study conducted
1.	H_1, H_2, H_3, H_4, H_5, H_6	Ceftiofur	2 $mg.kg^{-1}$	i.v.	Plasma levels, pharmacokinetics
2.	H_1, H_2, H_3, H_4, H_5, H_6	Ceftiofur	2 mg.kg-^{1}	i.m.	Plasma levels, pharmacokinetics
3.	H_1, H_2, H_3, H_4, H_5, H_6	Ceftiofur	2 $mg.kg^{-1}$	s.c.	Plasma levels, pharmacokinetics
4.	P_1,P_2,P_3	Untreated			*In vitro* plasma protein binding (PPB) of ceftiofur
5.	F_1, F_2, F_3, F_4, F_5, F_6	*E.coli*	1 $\mu g.kg^{-1}$	i.v.	Plasma levels, pharmacokinetics
		Ceftiofur	2 $mg.kg^{-1}$	i.v.	*In vitro* PPB of ceftiofur
6.	HP_1, HP_2, HP_3, HP_4, HP_5, HP_{6F}	Paracetamol	Day 0-250 $mg.kg^{-1}$	i.m.	Plasma levels, pharmacokinetics
		Ceftiofur	Day 2, 4-50 $mg.kg^{-1}$	i.v.	*In vitro* PPB of ceftiofur
			2 $mg.kg^{-1}$		
7.	_____	Pharmacodynamics of ceftiofur		_____	

estimation of plasma levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma Glutamyl Transferase (GGT), Total and Direct Bilirubin, Cholesterol, Albumin, Alkaline phosphatase (ALP) and Amylase.

A. Pharmacokinetics of ceftiofur in healthy buffalo calves.

Six healthy male buffalo calves were used to study the detailed pharmacokinetics of ceftiofur after its single i.v. administration. Freshly dissolved ceftiofur sodium (1 g in 20 ml sterile water) was administered at the dose rate of 2 $mg.kg^{-1}$ B.W. To investigate the kinetic pattern of the antibiotic by i.m. and s.c. route,

same dose of drug was injected in a cross over study, in which intravenous study was conducted, with a washout period of 3 weeks between two administrations. *In vitro* protein binding of ceftiofur was also studied in the blank plasma of healthy animals.

B. Pharmacokinetics of ceftiofur in febrile buffalo calves.

In above mentioned buffalo calves, fever was induced following single/repeated intravenous administration of *E. coli* endotoxin at the dose rate of 1 $\mu g.kg^{-1}$ B.W. Once fever was induced as observed by increase in the rectal temperature of animals by 1-2 ^0F, ceftiofur was administered at the dose rate of 2 $mg.kg^{-1}$ B.W. by i.v. route to study the pharmacokinetics. The *in vitro* protein binding of ceftiofur was also studied in the blank plasma of febrile animals.

C. Pharmacokinetics of ceftiofur in hepatic dysfunctioned buffalo calves.

After a washout period of 3 weeks in the buffalo calves, liver dysfunction was induced by intramuscular administration of paracetamol as already described. Ceftiofur was administered in these calves at the dose rate of 2 $mg.kg^{-1}$ B.W. by i.v. route to study the pharmacokinetics. The *in vitro* protein binding of ceftiofur was also studied in the blank plasma of hepatic dysfunctioned buffalo calves.

To study *in vitro* plasma protein binding, the blood samples from animals of group 3, 5 and 6 were collected before administration of ceftiofur.

Estimation of biochemical parameters in hepatic dysfunctioned buffalo calves:

To study the biochemical parameters, blood samples were collected in heparinized vials from the jugular vein of hepatic dysfunctioned animals on 0, 1, 2, 3, 4, 5 and 6^{th} day from the start of paracetamol administration. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20 ^0C till further analysis. Biochemical parameters including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Albumin, Alkaline phosphatase (ALP) and Amylase were

estimated using Bayer Autopack kits (Bayer Diagnostics India Ltd. Baroda, Gujarat) and Gamma Glutamyl Transferase (GGT) were estimated using Gamma-GT FS (Diasys Diagnostic Systems GmbH, Holzheim, Germany), Cholesterol was estimated using Erba diagnostic kits (Transasia Biomedicals Ltd. Mumbai).

Collection, processing and storage of samples

Ceftiofur sodium (Ceftivet, Pfizer India Ltd) was administered through jugular vein, into deep gluteal muscles of hindquarters and below the skin in the neck region of buffalo calves for intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.) route, respectively. The dose of ceftiofur administered in the present study was comparable to that used in previous studies in cattle (Brown *et al* 1996, Brown *et al* 2000 and Okker *et al* 2002). Blood samples (4-5 ml) were collected in tubes containing 8-10 units heparin per ml. of blood via a jugular venipuncture (using contra lateral vein from that to which the i.v. dose was administered) at 2.5, 5, 10, 15, 30, 45 min,1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24, 36 and 48 hours following ceftiofur administration. Plasma was separated at room temperature after centrifugation at 3000 rpm for 15 minutes and stored at -20^{o}c until further analysis.

3.5 Assay procedure for ceftiofur

Plasma concentrations of ceftiofur were determined using high performance liquid chromatography (HPLC) as per the modified method of Baere *et al* (2004) and Jacobson *et al* (2006). Both the assays followed are modifications of the original method reported by Jaglan *et al* (1990) for the quantitative determination of ceftiofur and its active metabolite desfuroylceftiofur (DFC) in plasma during pharmacokinetic study, but Baere and co-workers (2004) stated that their assay was useful for the quantitative estimation of both free and bound the antibiotic.

3.5.1 Instrumentation and chromatographic conditions

HPLC system (Perkin Elmer) consisted of a single pump (Perkin Elmer series 200), a degasser (Perkin Elmer series 200) and an autosampler injector with 200 microliter loop (Perkin Elmer series 200). The detection was performed using an UV/VIS detector (Perkin Elmer 200 series) set at 265 nm. The reverse-phase chromatography was performed with an analytical C_{18} column (Lichrosphere®, Particle size 5µ, 4.6 × 150 mm, Merck, Germany). Gradient phase conditions consisted of 100% solvent A (0.1% TFA in water) decreasing linearly to 75% solvent A, 25% solvent B (0.1% TFA in acetonitrile). The retention time of the derivatized desfuroylceftiofur acetamide was 22.5–23.5 min, with a total run time of 25.0 min, and equilibration time of 2.0 min. Mobile phase was filtered, degassed by passage through a 0.22 µm nylon filter (Millipore, Bedford, MA) under vacuum and sonicated for 30 min. The flow rate was 1 $ml.min^{-1}$ and the injection volume was 50 µl. TotalChrom software® (version 6.1) was used for instrument control and data analysis.

3.5.2 Stock solutions and standards

A standard solution of ceftiofur was prepared by the weighing and subsequent dissolution in Dimethyl Sulfoxide (DMSO). The concentration of the standard stock solution was 2 $mg.ml^{-1}$. The primary stock solution of ceftiofur was diluted quantitatively with water for the preparation of calibration standards and quality control (QC) samples. Ceftiofur calibration standards were prepared fresh daily at concentrations of 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 1, 5 and 10 $\mu g.ml^{-1}$ by spiking 450 µl blank buffalo calf plasma with 50 µl of water (for the "zero" standard sample) or ceftiofur working solutions. In the same manner, QC samples with ceftiofur at low (0.1 $\mu g.ml^{-1}$), medium (5.0 $\mu g.ml^{-1}$), and high (15 $\mu g.ml^{-1}$)

concentrations were freshly prepared to evaluate the accuracy and precision of this HPLC method. Prior to HPLC analysis, these calibration standards and QC samples were processed according to the method that is outlined as follows.

3.5.3 Sample preparation procedure

To 500 µl of plasma, 7ml of the extracting solution (0.4% dithioerythritol in borate buffer) was added. After vortexing, the tubes were placed in a shaking water bath (moderate speed) at 50 ^{0}C for 15 min. The samples were cooled down to room temperature and 1.5 ml of the iodoacetamide solution (14% iodoacteamide in phosphate buffer pH 7.4) was added. The samples were mixed well and were kept in the dark at room temperature for 30 min with intermittent vortexing. Following derivatization, the samples were transferred to an Oasis HLB SPE column (60 mg/3 ml) that was preconditioned with 1 ml of methanol and 1 ml of water. The sample was allowed to pass under gravity through the HLB column. Then the column was washed with 1 ml of 5% (v/v) methanol in water and dried by applying vacuum for 1-2 min. Elution was performed using 1 ml of a 5% (v/v) solution of acetic acid in acetonitrile. The eluate was concentrated to dryness under vacuum concentrator (Eppendorf 5301®) at 30 ^{0}C. The dry residue was dissolved in 500 µl of 0.1 % TFA in water, vortexed and filtered through 0.45 µm nylon filter (Millipore, Bedford, MA) and transferred to an autosampler vial. A 50 µl aliquot was then injected onto the HPLC-UV system.

3.6 Method Validation

3.6.1 Selectivity

Selectivity of the method was evaluated by analyzing blank buffalo calf plasma, plasma spiked with ceftiofur and plasma samples obtained from ceftiofur pharmacokinetic studies of the buffalo calves. For all plasma samples, chromatograms

were visually examined for chromatographic interference from endogenous compounds. The retention time of ceftiofur was about 23 ± 0.5 min, with a total run time of 27 min. Representative chromatograms of blank buffalo calf plasma, plasma sample spiked with 10 $\mu g.ml^{-1}$ of ceftiofur and in the buffalo calf plasma collected at 10 min after i.v. administration of ceftiofur at a dose of 2 $mg.kg^{-1}$ are shown in Fig.3, 4 and 5 respectively.

3.6.2 Calibration curve

The linearity of the method was evaluated by a calibration curve in the range of 0.1 to 10 $\mu g.ml^{-1}$ ceftiofur (figure 6). Calibration standards were run before and after the samples; both sets of standard peak areas were used to calculate the linear regression equation as well as the coefficient of determination. Blank samples were included with each set. Three calibration curves were constructed on three separate days and peak area of ceftiofur was plotted against the corresponding concentrations. The calibration curve was constructed by weighted (1/y) least- squares linear regression analysis. The calibration curves were described by the following linear equation: $y = ax \pm b$, where *y* is the analyte area, *a* is the slope of the calibration curve, b is the y intercept and x is the concentration ($\mu g.ml^{-1}$). The slope, intercept and correlation coefficient were calculated for each standard curve. The correlation coefficients (r), indicating the functional linear relationship between the concentration of analyte and the area under the peak was 0.9999 across the concentration range used. Unknown plasma concentrations were computed from the equation of the calibration curve.

3.6.3 Sensitivity

The limits of detection (LOD) and quantification (LOQ) were determined by signal-to-noise ratio evaluations of samples spiked from 0.01 to 0.2 $\mu g.ml^{-1}$. The LOD

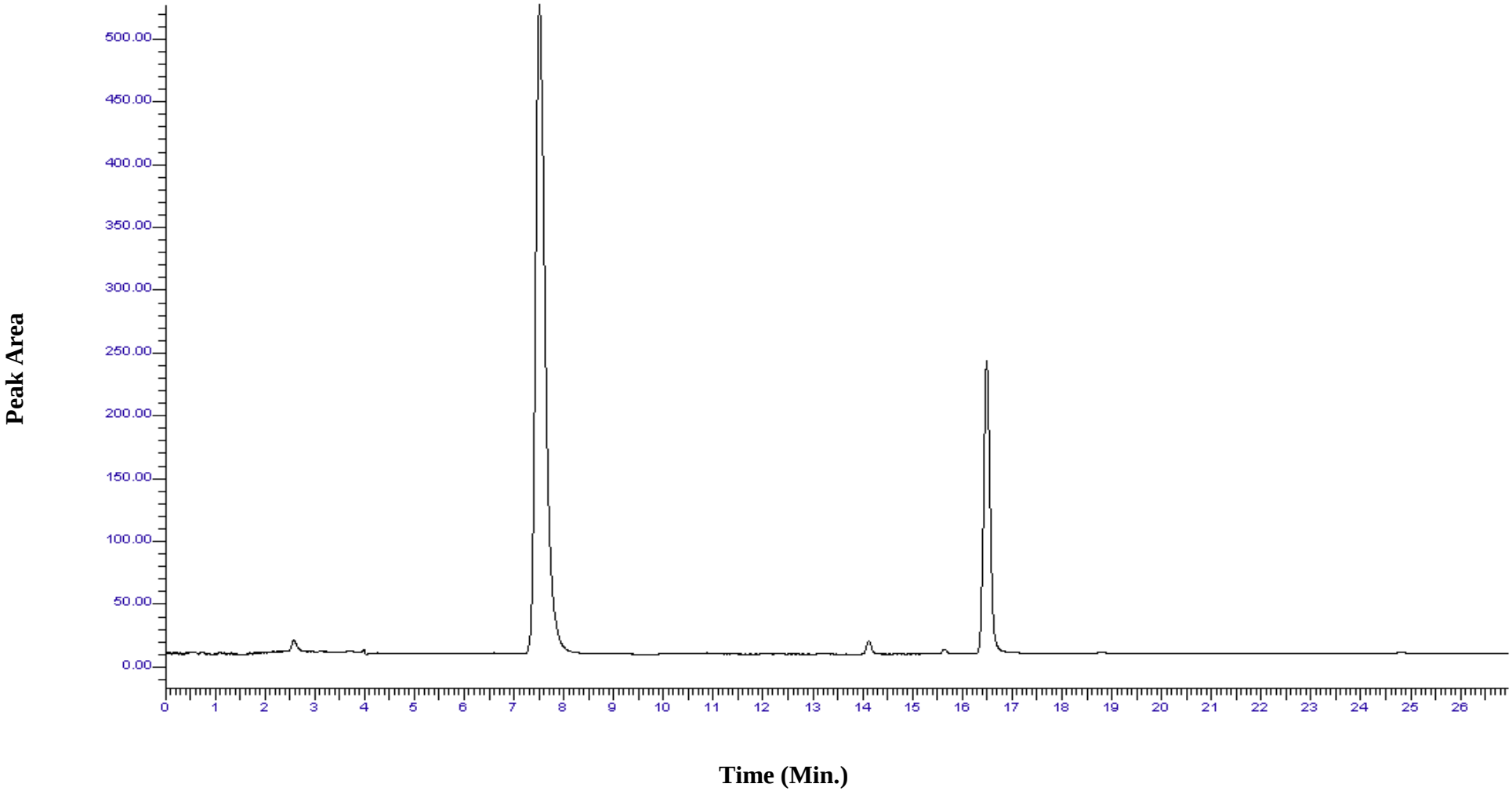


Fig. 3: Chromatogram of blank (unspiked) plasma sample

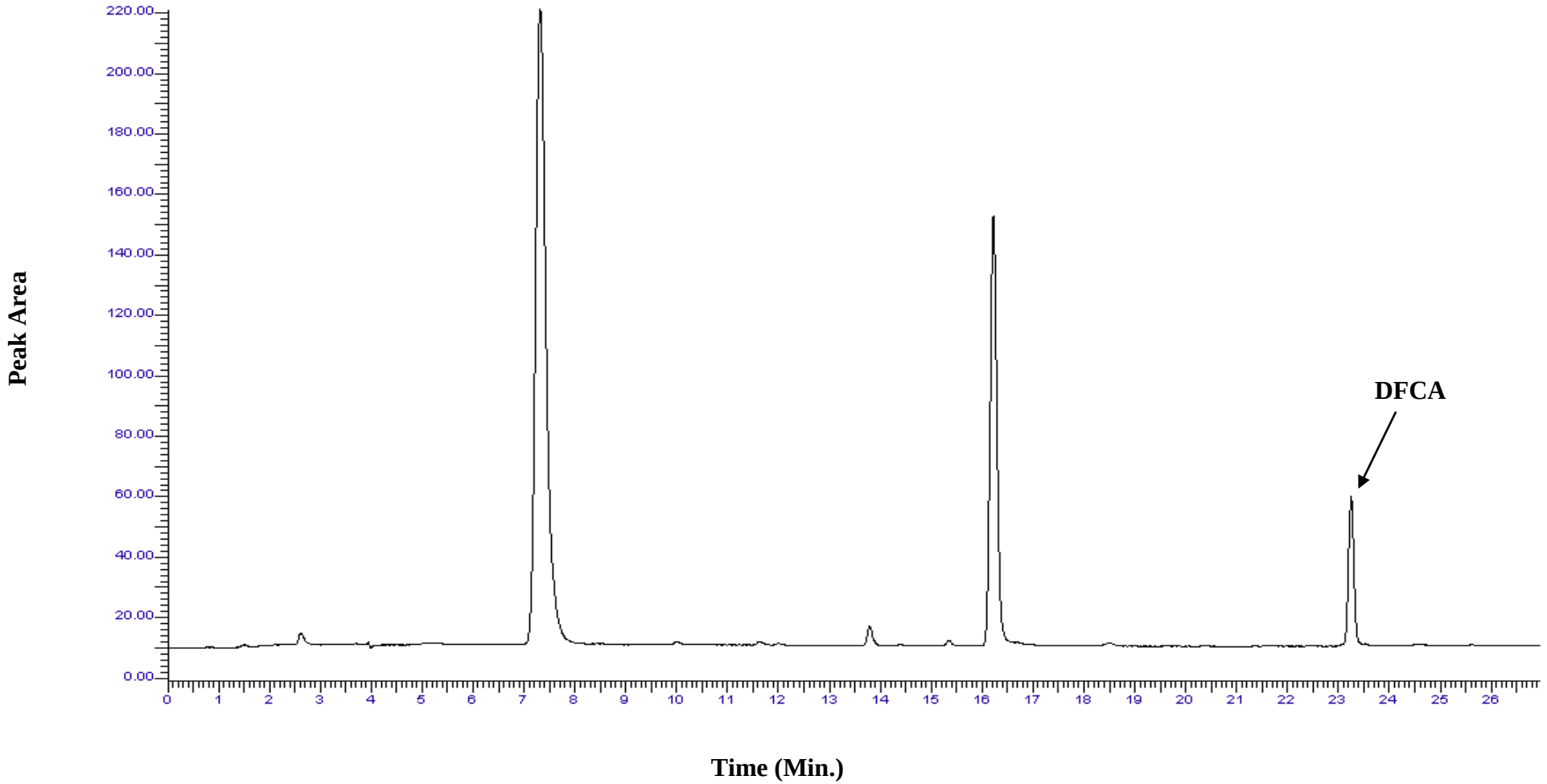


Fig. 4: Chromatogram of buffalo calf plasma sample spiked with ceftiofur (10 µg.ml^{-1})

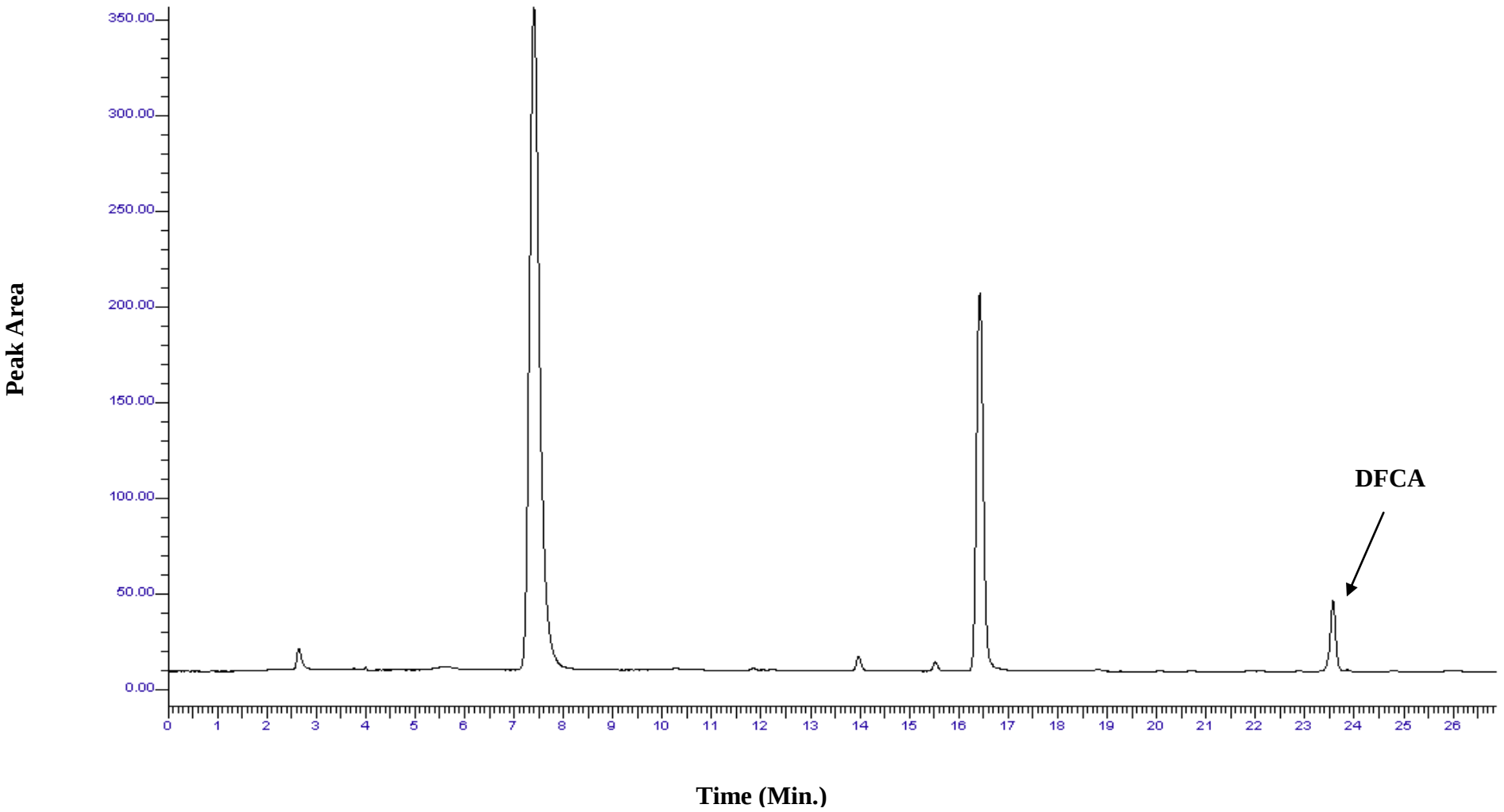


Fig. 5: Chromatogram of buffalo calf plasma sample 10 min after intravenous administration of ceftiofur sodium (2 mg.kg^{-1})

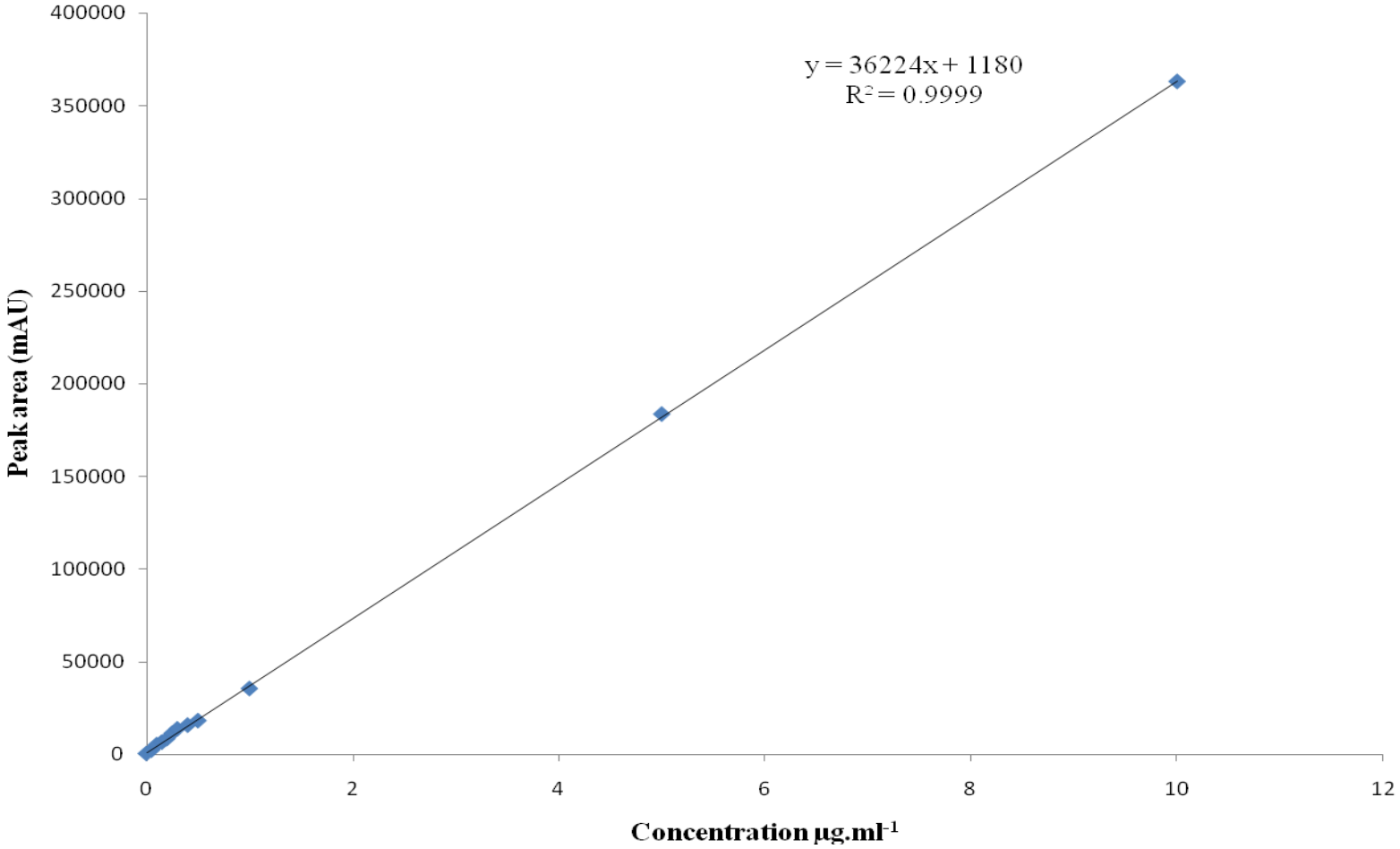


Fig. 6: Standard curve in plasma of buffalo calves spiked with ceftiofur

is a parameter that provides the lowest concentration in a sample that can be detected from background noise but not quantitated. The LOD was defined as the lowest concentration with a signal-to-noise ratio of at least 3. LOQ is defined as the lowest concentration of analyte with a signal-to noise ratio of at least 10 with acceptable accuracy (> 90%) and precision (<15%) for each criterion. The LOQ was established by determining the concentrations of four spiked calibration standards from 0.01 to 0.1 $\mu g.ml^{-1}$. The LOQ of the method was found to be 0.1 $\mu g.ml^{-1}$ for ceftiofur in buffalo calves plasma with acceptable accuracy and precision. The LOD was determined to be 0.03 $\mu g.ml^{-1}$, based on a signal-to-noise ratio of 3:1.

3.6.4 Precision and accuracy

Ceftiofur QC samples at low, medium, and high concentrations were spiked for the determination of precision and accuracy and prepared as described above. Three replicates of each level of QC samples were assayed in one run for the intraday experiment and three replicates of each level of QC samples were assayed within three different days for the interday experiment. The intra- and interday precision and accuracy of the assay were determined as percent coefficient of variation (CV) and percent bias values, respectively. The coefficient of variation was calculated as follows:

$$\text{CV (\%)} = (\text{standard deviation/mean}) \times 100.$$

The percent bias values from the theoretical concentration were calculated by the following equation:

$$\text{Bias (\%)} = \frac{\text{Calculated concentration} - \text{Theoretical concentration}}{\text{Theoretical concentration}} \times 100$$

Acceptance criteria for accuracy and precision were as follows: the CV was lower than 5% and the bias was within ±5%. Inter-and intraday precision, in terms of

the CV, was obtained by subjecting the data to one-way analysis of variance (ANOVA). The accuracy and precision of the method were evaluated with QC samples at concentrations of 0.1, 1 and 10 $\mu g.ml^{-1}$. At all levels, % coefficient of variation was lower than 5%. The accuracy levels were > 95%. These results indicated that the proposed method was precise and accurate.

3.6.5 Recovery

For calculation of absolute recoveries of ceftiofur, spiked QC samples were prepared at low (0.1 $\mu g.ml^{-1}$), medium (5 $\mu g.ml^{-1}$) and high (10 $\mu g.ml^{-1}$) concentrations. Six replicates of each QC sample were extracted by the afore-mentioned sample preparation method and injected into the HPLC system. The concentration of ceftiofur was determined from the linear regression of the analytical standard curve. The absolute recovery was calculated by comparing the peak areas of the prepared QC samples with those of the standard solutions. The extraction recoveries of ceftiofur from plasma were in the range of 90 – 105% for the low, medium and high QC samples. The recovery of ceftiofur using the described procedure was consistent and efficient.

3.7 Quantification

The regression formula obtained from the calibration curves was used to quantify the concentration of ceftiofur in plasma by substituting respective analyte area.

$$y = ax \pm b$$

where,

y = analyte area

b = y intercept

a = slope of the calibration curve

x = concentration ($\mu g\ ml^{-1}$)

3.8 Pharmacokinetics analysis

The best suited model for pharmacokinetic analysis was determined by visual examination of individual concentration–time curves. The peak plasma concentration (C_{max}) and the time to achieve C_{max} (T_{max}) after parenteral administration were estimated from plasma concentrations from all animals. Various pharmacokinetic determinants were calculated from the plasma concentration-time profile of ceftiofur for each animal according to standard equations given by Gibaldi and Perrier (1982). The regression lines for absorption/distribution and elimination phases were drawn, and the following pharmacokinetic parameters were calculated as per the method described by Srivastava and Bal (1994).

1. β, the overall elimination rate constant

   $\beta = 2.303 \times m$

   $$m \text{ (regression coefficient)} = \frac{\sum XY - \sum X \sum Y}{\sum X^2 - \frac{\left(\sum X\right)^2}{n}}$$

   m was calculated by the method of "least square technique."

   B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase.

   $B' = \text{Antilog } (mx + c)$

   B = Antilog of C

2. Ka and A', are hybrid rate constants associated with absorption of drug and zero time plasma drug concentration intercept of regression line of absorption phase, respectively. Ka and A' were calculated by the regression analysis.
3. α_1 and α_2 are hybrid rate constant associated with early and late distribution of drug from central compartment whereas A_1 and A_2 are zero time plasma drug

concentration intercept of regression lines of respective distribution phases. α_1, α_2, A_1 and A_2 were calculated by the "Feathering Technique."

4. $t_{½}ka$, $t_{½}\alpha 1$, $t_{½}\alpha 2$ and $t_{½}\beta$ are absorption, distribution and elimination half-lives, respectively.

$$\text{Half-life} = \frac{\ln 2}{\text{Rate constant(s)}} = \frac{0.693}{\text{Rate constant(s)}}$$

5. Area under curve, $AUC_{0-\infty}$, total area under the curve from 0 - ∞

$AUC_{0-\infty} = AUC_{0-tL} + AUC_{tL-\infty}$ (Trapezoidal method)

where,

AUC_{0-tL} = Area under the curve up to last sampling

$AUC_{tL-\infty}$ = Area under the curve beyond last sampling

$AUC_{0-tL} = \Sigma ½(C_{n-1} + C_n).(t_n - t_{n-1})$

where,

t_n = time of n^{th} sample collection

t_{n-1} = time of $n\text{-}1^{th}$ sample collection

C_n = Concentration of ceftiofur at t_n^{th} time

C_{n-1} = Concentration of ceftiofur at t_{n-1}^{th} time

$AUC_{tL-\infty} = C_L/\beta$

where,

C_L = Concentration of ceftiofur at last sample collection

β = Slope of terminal phase of elimination

6. Area under moment curve, $AUMC_{0-\infty}$ total area under first moment curve from 0-∞

$AUMC_{0-\infty} = AUMC_{0-tL} + AUMC_{tL-\infty}$

where,

$AUMC_{0\text{-}tL}$ = Area under moment curve up to last sampling

$AUMC_{tL\text{-}\infty}$ = Area under moment curve beyond last sampling

$AUMC_{0\text{-}tL} = \Sigma ½(C_{n-1}.t_{n-1} + C_n t_n).(t_n - t_{n-1})$

where,

$C_n t_n$ = Product of ceftiofur concentration and time at n^{th}collection.

$C_{n-1}.t_{n-1}$ = Product of ceftiofur concentration and time at $n\text{-}1^{th}$ collection.

t_n and t_{n-1} = time of n^{th} and $n\text{-}1^{th}$ sample collection, respectively.

$AUMC_{tL\text{-}\infty} = (t_L.C_L)/\beta.(C_L)^2/\beta^2$

where,

C_L = ceftiofur concentration ($\mu g.ml^{-1}$) at last sampling.

t_L = time of last sampling.

β = slope of terminal phase of elimination.

7. V_d, the apparent volume of distribution

   a. $V_{d(area)}$, based on the total area under the plasma drug concentration versus time curve.

      i. $V_{d(area)} = \frac{\text{Dose } (mg.kg^{-1})}{\beta.AUC_{0\text{-}\infty}}$

   b. $V_{d(B)}$, based on zero-time plasma concentration intercept of the least square regression line of elimination phase (extrapolation method; Nelson 1961).

      i. $V_{d(B)} = \frac{\text{Dose}}{B}$

   c. $V_{d(SS)}$, based on average steady state plasma level

      i. $V_{d(SS)} = Cl_B \times MRT$

8. 9. Cl_B, the total body clearance of drug.

$Cl_B = \beta.V_{d(area)}$ X 1000

9. 10. The time for which the plasma drug levels remain above or equal to minimum inhibitory concentration (MIC) value is calculated using the formula:

$$\%T > MIC = \ln \{D / Vd_{area} \times MIC\} \times \{t_{1/2}\beta / \ln(2)\} \times \{100 / DI\}$$

Where, T > MIC is the time interval (in percent) during which the plasma concentration is above or equal to the MIC values, ln is natural logarithm, D is the proposed dose, Vd_{area} is the volume of distribution, $t_{1/2}\beta$ is the terminal elimination half-life and DI is the dose interval (Turnidge 1998). The definitions of various pharmacokinetic parameters are presented in table 2.

Table 2: Definition of PK parameters

Parameters	**Unit**	**Definition**
A', A, B	$\mu g.ml^{-1}$	Zero time intercepts of regression lines of absorption, distribution and elimination phases, respectively
Ka, α, β	h^{-1}	Absorption, distribution and elimination rate constants, respectively.
t½ Ka, t½α, t½ β	h	Absorption, distribution and elimination half lives, respectively.
AUC	$\mu g.h.ml^{-1}$	Total area under the plasma drug concentration time curve.
AUMC	$\mu g.h^2.ml^{-1}$	Total area under the first moment of plasma drug concentration time curve
Vd	$L.kg^{-1}$	Apparent volume of distribution: volume of body fluids and tissues having the same concentration of drug as in blood in a unit time.It is calculated by various methods and is represented as: Vd_{area}, based on total area under the plasma drug concentration time curve Vd_B, based on the zero time plasma drug concentration intercept of elimination phase Vd_{ss}, based on average steady state plasma level
F	%	Per cent of drug available in the central compartment after extravascular administration
Cl_B	$L.kg^{-1}h^{-1}$	Total body clearance of the drug

3.9 *In vitro* plasma protein binding of ceftiofur

In vitro binding of ceftiofur to plasma proteins was determined by employing the equilibrium dialysis technique (Gupta *et al* 2006). The dialyzing bags (4 Å pore size), 10 cm long were washed in running tap water and soaked overnight in phosphate buffer. Ceftiofur concentrations 1, 2, 4, 5 and 10 µg ml^{-1} were prepared in pooled plasma separated from blood taken from untreated, febrile and hepatic dysfunctioned animals.

Each dialyzing bag was knotted at one end before filling 5 ml of plasma containing known amount of drug and the other end was then securely tied. Each bag was immersed in separate tubes containing 5 ml of phosphate buffer (0.2 M; pH 7.4; disodium hydrogen phosphate 11.3 g, potassium-dihydrogen phosphate 2.7 g, added to 1000 ml of distilled water) and the tubes were incubated at 37^0C for 24 h with intermittent shaking. At the end of incubation period phosphate buffers as well as contents of the dialyzing bags were separately analyzed for the concentration of ceftiofur by HPLC assay described above. The extent of *in vitro* plasma protein binding of ceftiofur was calculated by the following equation.

$$\text{Percent of ceftiofur bound to plasma protein} = \frac{CP' - CB}{CP} \times 100$$

where, CP' = Concentration of ceftiofur in plasma after incubation.

CB = Concentration of ceftiofur in buffer after incubation.

CP = Concentration of ceftiofur in plasma before incubation.

Binding capacity of the plasma protein to ceftiofur (β_i) and the dissociation rate constant of protein drug complex (K_β) were calculated by the method of Pilloud (1973).

3.10 Pharmacodynamics of ceftiofur

The PD parameters *viz*., MIC and MBC of ceftiofur against *E. coli* and *S. aureus* were determined in Muller Hinton broth (MHB) using broth microdilution

method according to CLSI guidelines (CLSI 2008) (Sidhu *et al* 2010).

3.10.1 Requirements

Test Organism *E. coli* (MTCC 739 reference strain) and *S. aureus* (MTCC 737 reference strain) were obtained from IMTECH Chandigarh.

37.5 g EMB Agar (Hi-media Laboratories Pvt. Ltd. Mumbai) and 111 g Mannitol Salt Agar (Merck India Pvt. Ltd. Mumbai) was added separately to 1 L of sterile distilled water, and then sterilized by autoclaving at 121^0C for 15 min. 15 lbs pressure. After sterilization the media was transferred to a water bath at 37°C. The liquid media were poured into sterile petri plates and cooled to room temperature. The plates were stored in refrigerator at 4^0C after checking for sterility. Mueller-Hinton broth, (Himedia Laboratories Pvt. Ltd. Mumbai): 21 g of the media was added to 1 litre of sterile distilled water, and then autoclaved at 121°C for 15 min. The broth was stored at 4ºC for 1-2 weeks. Other materials like eppendorf tubes, micro-pipette tips were sterilized by autoclaving at 121ºC for 15 min. 15 lbs pressure.

3.10.2 Standardization of inoculum size

Two medium sized colonies of *E. coli* and *S. aureus* grown on EMB agar and Mannitol salt agar (MSA) were inoculated in a separate test tubes contains 9 ml of MHB and incubated at 37°C for 4 h and 8 h respectively. After incubation the turbid broths were diluted to 10^{-2}, 10^{-4} and 10^{-6} using fresh MHB. After thorough mixing of diluted broth, EMB and MSA plates were inoculated in triplicates with 10 µl broth from each dilution and incubated at 37°C for 18 hrs. The growth was observed and colonies of *E.coli* and *S.aureus* were counted visually. For example:

Number of colonies forming units per ml (CFU/ml) = 500 with dilution 10^{-6}.

Viable count = 5×10^8 CFU/ml.

3.10.3 Determination of MIC

The MHB were spiked to concentrations of 0.01 -1 µg.ml^{-1} of ceftiofur in microwell plates using working standard solutions of ceftiofur (0.2- 20 µg.ml^{-1}). The concentrations were selected to cover the most probable points of expected MIC. To these wells 10 µl of bacterial cultures of *E. coli* and *S. aureus* with inoculum size of 1 – 5 x10^{8} CFU/ml were added, mixed and incubated at 37°C for 18 h. Growth controls were run with MHB without addition of ceftiofur. The final volume of the mixture was 200 µl. After incubation, drug concentrations at which there was minimum visible bacterial growth or turbidity were selected and plated on to the EMB and mannitol salt agar plates respectively for *E.coli* and *S.aureus* using 10 µl from each dilution (original inoculum, 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8}). Once the drops had dried, plates were incubated at 37 ºC for 18 h before counting. Results were calculated as counts per ml and limit of detection was 10 CFU/ml. Growth controls were plated for comparison. Minimum inhibitory concentration was the lowest concentration of the antibiotic at which bacterial count remained below the original inoculums level. The MIC of ceftiofur was determined as the lowest concentration of drug that completely inhibits the visible growth of the *E. coli* as well as *S. aureus* (detected by the absence of turbidity) compared to the growth control. The MIC was performed in five over-lapping sets in the range of 0.01 – 1 µg.ml^{-1} of ceftiofur to increase the accuracy of MIC.

3.10.4 Determination of MBC

The MHB was spiked with ceftiofur concentrations of 0.05, 0.10, 0.20, 0.50, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 µg.ml^{-1} in flat bottom tissue culture plates. The concentrations were selected to cover the most probable points of expected MBC, which is ≥ MIC. To these wells 10 µl of broth containing bacterial cultures of *E. coli*

and *S. aureus* with inoculum size of 5 x10^8 CFU/ml were added, mixed and incubated at 37°Cfor 18 h. Growth controls were used with MHB without addition of ceftiofur. The final volume of the mixture was 1000 µl. After incubation, drug concentrations at which there was no visible growth or turbidity were selected and plated on to the EMB and blood agar plates respectively for *E .coli* and *S. aureus* using 10 µl from each dilution (original inoculum, 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8}). Once the drops had dried, plates were incubated at 37 ºC for 18 h before counting. Results were calculated as counts per ml and limit of detection was 10 CFU/ml. Growth controls were plated for comparison. A MBC was recorded as the lowest drug concentration which reduced bacterial numbers by 99.9% of the original inoculums (reduction of 1000 fold of initial inoculums) i.e. 10^5 CFU/ml.

3.11 Statistical analysis

The differences between two means based on individual observation were determined by Student's t-test and ANOVA using SPSS® 16.0 software package. The significance was assessed at $P < 0.05$ and $P < 0.01$ level (Singh *et al* 1991).

CHAPTER IV

RESULTS AND DISCUSSION

The common motive of drug administration to an animal is to produce a certain pharmacological response. This criterion may be achieved, at least in part, by giving a recommended dose of the drug. To produce its characteristic effect(s), a drug must be present in appropriate concentrations at its site of action. When a drug is given at the same dose rate, wide variations in intensity as well as duration of pharmacological effect are usually observed among species of domestic animals.

The basic principle of judicious and successful use of an antimicrobial completely depends upon its dosage regimen, route of administration and also the disease status of animals. The ineffectiveness of an antimicrobial against an infection might be because of its inability to achieve appropriate minimum concentration at site of infection when administered at selected dose. Toxicity may occur if the drug gets accumulated in the body when administered at higher dose. Therefore for the rational antibiotic therapy of an antibiotic, dosage regimens should be optimized in the species in which it is to be used clinically so as to minimize risk of emergence of resistance.

To study the disposition pattern of an antimicrobial in any species, it is to be administered by single intravenous, intramuscular and subcutaneous routes. So, in order to use ceftiofur judiciously in buffalo species, it was considered practical, to determine its pharmacokinetics by different routes i.e. i.v., i.m. and s.c. in healthy buffalo calves. Pharmacokinetics of antimicrobials may be altered in disease conditions. Therefore to optimize dosage regimen in very common clinical conditions, pharmacokinetics of ceftiofur was investigated in febrile and hepatic dysfunctioned buffalo calves.

Antimicrobials are essential for the treatment of animal diseases and thus play a key role in livestock production. Antibiotics are being used extensively and without

any need in veterinary practice thus posing the risk of antimicrobial resistance (Tenover and Hughes 1996 and Akkina *et al* 1999). Antimicrobial resistance is defined as a property of bacteria that confers the capacity to inactivate or exclude antibiotics or a mechanism that blocks the inhibitory or killing effects of antibiotics, leading to survival of microbes despite exposure to antimicrobials (National Research Council, Institute of Medicine, 1998). The term multi-drug resistant is used when some bacteria shows resistance against different groups of antibiotics. The international human and animal health communities, medical and veterinary clinicians are very much concerned about the loss of effectiveness of antimicrobials due to their indiscriminate use and development of resistance. This issue has the potential to affect livestock economy through impact on animal welfare through changes in the makeup of the food supply, cost and overall food quality. Increasing reports of human and animal outbreaks associated with multi-drug resistant pathogens have alarmed the scientific community about the transfer of antibiotic resistance from animals to humans (Akkina *et al* 1999). To reduce the risk of resistance, few strategies being adopted worldwide these include limiting antibiotics availability, monitoring and surveillance programs and on farm interventions at the local level. These on farm interventions comprise prudent use of antimicrobials along with implementation of management practices which decrease the need for antimicrobials. Prudent use of antimicrobials is defined as the use in a manner that promotes their effectiveness and minimizes bacterial resistance development (Apley *et al* 1998 and Huovine and Cars 1998). Identification of the specific viral or bacterial infection using new diagnostic tools, determination of antibiotic susceptibility to the target pathogen and development of species specific optimum dosage schedules to augment efficacy on the basis of PK-PD studies can facilitate prudent use of antimicrobials. In recent few

years, several researchers have elucidated the dynamic relationship between PK and PD variables (Aliabadi *et al* 2003 and Peggy *et al* 2008). In the present investigation, PK and PD characteristics of ceftiofur have been determined in buffalo species to establish optimal dosage regimen in terms of dose and dosing interval as an attempt to prevent the emergence of microbial resistance against organisms of veterinary importance.

(A) Pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in healthy buffalo calves

1. Plasma levels and Pharmacokinetics

(i) Intravenous administration

To study the disposition kinetics of ceftiofur in buffalo calves, the drug was injected as a single dose of 2 $mg.kg^{-1}$ body weight by i.v. route. Intravenous administration of antibiotic is obligatory mean for treatment of severe bacterial infections and managing health of critically ill animals. For obvious reasons, it is very much essential for treating bacterimia, meningitis and shock, since very high initial antibiotic concentration in central compartment is required to obtain a favourable diffusion gradient from blood to tissues.

Ceftiofur after administration, regardless of route, gets rapidly hydrolysed to microbiologically active metabolite desfuroylceftiofur (DFC) and furoic acid. DFC is further metabolized to its cysteine disulfide which gets bound to proteins present in the tissue and body fluids (Beconi-Barker *et al* 1995). It has been reported that conversion of ceftiofur to active metabolite DFC occurs within one hour of either intravenous (i.v.) or intramuscular (i.m.) administration in cattle so that no parent drug was detected after one hour of the treatment (Brown *et al* 1991 and Jaglan *et al* 1990). As free DFC is microbiologically active (Baere *et al* 2004), the activity and efficacy

of ceftiofur largely depend on the binding of DFC and it's disulfide conjugate to the macromolecules in plasma and tissues (Jaglan *et al* 1994). Method that quantitates only the parent compound usually underestimates the microbiological activity related to the *in vivo* administration of this drug. So a method that determines both the free and bound form of ceftiofur and its active metabolite DFC, is considered to be suitable for the quantification in a pharmacokinetic study (Baere *et al* 2004). This estimation was therefore carried out by derivatisation with iodoacetamide to form desfuroylceftiofur acetamide (DFCA).

The literature suggest that the pharmacokinetics of ceftiofur in small ruminant species i.e. sheep and goats were similar to that seen in cattle (Craigmill *et al* 1991 and Courtin *et al* 1997). The dose of ceftiofur sodium selected in the present study was similar to that used in previous studies in cattle (Brown *et al* 1996, 2000, Okker *et al* 2002, Tohamy 2008 and Liu *et al* 2010).

The plasma levels of ceftiofur and desfuroylceftiofur after single i.v. administration of ceftiofur sodium (2 $mg.kg^{-1}$ B.W.) in healthy buffalo calves are presented in table 3. Parent drug and its metabolite were estimated up to 36 h post administration in the plasma of buffalo calves. Evaluation of semi logarithmic plot of mean plasma ceftiofur and DFC concentration versus time curves following i.v. administration was best fitted into a three compartment open model (Fig 7). The triexponential equation describing model for i.v. route was

$$C_P = A_1e^{-\alpha 1t} + A_2e^{-\alpha 2t} + Be^{-\beta t}$$

Where, C_P is the plasma concentration of ceftiofur and DFC at time 't'. A_1, A_2 and B are zero time intercepts of early distribution, late distribution and elimination phase of log concentration time curve, respectively. The α_1, α_2 and β are the early distribution, late distribution and elimination rate constants, respectively.

Table 3: Plasma levels (μg.ml^{-1}) of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Time (h)	Animals						Mean ± SE
	1	2	3	4	5	6	
0.04	10.4	11.3	11.2	11.9	10.8	11.0	11.1 ± 0.21
0.08	9.35	9.18	9.45	9.12	9.65	8.98	9.29 ± 0.10
0.17	7.56	8.16	8.15	7.98	7.87	7.58	7.89 ± 0.11
0.25	7.16	7.33	7.25	7.27	7.65	6.84	7.25 ± 0.11
0.50	6.26	5.82	6.52	5.95	5.91	6.23	6.12 ± 0.11
0.75	4.58	4.51	5.14	4.52	4.74	5.14	4.77 ± 0.12
1.00	3.82	3.30	3.88	3.58	3.39	3.59	3.59 ± 0.09
2.00	2.20	2.34	2.38	2.08	2.24	2.43	2.28 ± 0.05
3.00	1.75	1.75	1.69	1.56	1.70	1.58	1.67 ± 0.03
4.00	1.51	1.45	1.12	1.21	1.41	1.41	1.35 ± 0.06
5.00	1.26	1.17	1.10	1.05	1.19	1.04	1.13 ± 0.03
6.00	1.10	0.91	0.97	0.89	1.02	0.95	0.97 ± 0.03
8.00	0.74	0.67	0.69	0.73	0.78	0.75	0.73 ± 0.02
10.0	0.62	0.59	0.50	0.57	0.65	0.51	0.57 ± 0.02
12.0	0.52	0.48	0.42	0.45	0.56	0.43	0.48 ± 0.02
16.0	0.41	0.35	0.30	0.36	0.43	0.37	0.37 ± 0.02
24.0	0.29	0.25	0.19	0.21	0.23	0.22	0.23 ± 0.01
36.0	0.12	0.10	0.14	0.11	0.13	0.14	0.12 ± 0.01

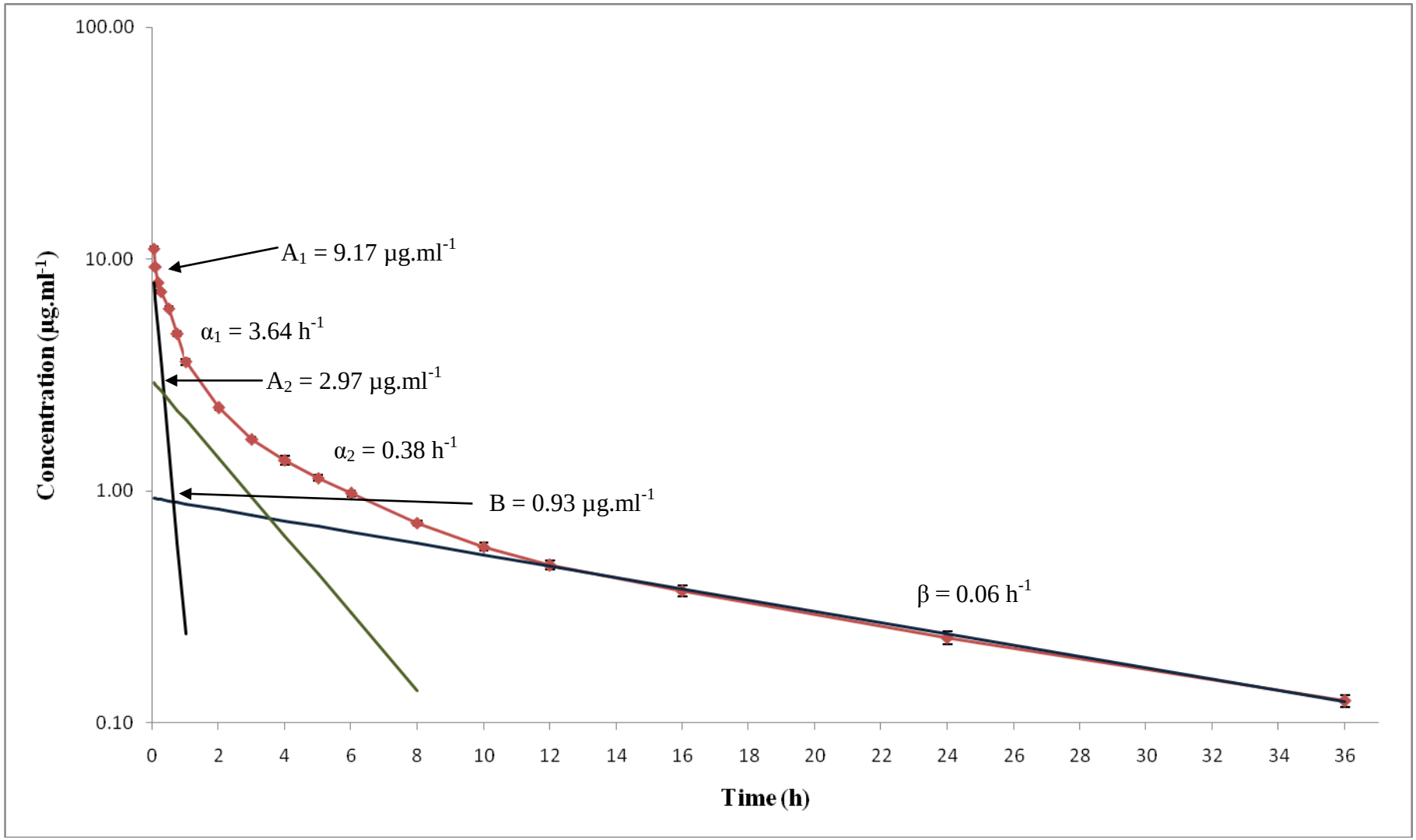


Fig. 7: Semilogarithmic plot of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

The log DFCA concentration versus time data for each buffalo calf after i.v. administration was different from literature and best fitted individually into a three-compartment open model. Previous pharmacokinetic studies recorded two compartment model in calves (Brown *et al* 1996) cattle (Whittem *et al* 1995), dairy goats (Courtin *et al* 1997), sheep (Craigmill *et al* 1997 and cows (Tohamy 2008 and Liu *et al* 2010) following i.v. dosing.

Pharmacokinetic parameters for ceftiofur and its metabolite after single intravenous administration of ceftiofur sodium (2 $mg.kg^{-1}$ B.W.) in healthy buffalo calves are depicted in table 4. Distribution half life ($t_{½}\alpha1$ = 0.19 ± 0.008 h and $t_{½}\alpha2$ = 1.84 ± 0.11 h) in the present investigation was longer denoting better persistence of ceftiofur and its metabolite DFC in the tissue and extracellular fluid in buffalo calves following i.v. dosing. The values for distribution half lives seen in bull calves (0.74 h), non lactating goats (0.8 h), cows (0.462 h, 1.3 h) after i.v. administration were much lower than present ($t_{½}\alpha2$) study (Brown *et al* 1996, Courtin *et al* 1997, Tohamy 2008 and Liu *et al* 2010).

The value of area under curve (AUC) indicates the plasma exposure of drug in the body and thus its antimicrobial activity against susceptible pathogens. Observed AUC in the present study (27.4 ± 0.48 $\mu g.h.ml^{-1}$) was similar to that reported in goats (33.93 $\mu g.h.ml^{-1}$) after i.v. dosing (Courtin *et al* 1997). However, much greater AUCs (72.4, 68.5, 88.0, 163 $\mu g.h.ml^{-1}$) were observed in cattle, bull calves and cows respectively (Whittem *et al* 1995, Brown *et al* 1996, Tohamy, 2008 and Liu *et al* 2010). The variation in the values might be due to difference in the body fluids among different species at various ages along with use of long acting crystalline free acid equivalent (CFAE) preparation. Microbial assay method for estimation of ceftiofur followed in some of these studies might not be as sensitive for estimation of ceftiofur and DFC as compared to the method used in the present investigation.

Table 4: Pharmacokinetic parameters of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Parameter (Unit)	Animals						Mean ±SE
	1	2	3	4	5	6	
α_1 (h^{-1})	3.33	3.70	3.32	3.95	3.34	4.20	3.64 ± 0.15
A_1 (µg.ml^{-1})	9.38	8.21	9.10	10.1	9.35	8.81	9.17 ± 0.26
$t_{½}$ α_1 (h)	0.21	0.19	0.21	0.18	0.21	0.16	0.19 ± 0.008
α_2 (h^{-1})	0.33	0.48	0.33	0.40	0.39	0.38	0.38 ± 0.02
A_2 (µg.ml^{-1})	2.31	3.87	2.99	2.70	2.68	3.30	2.97 ± 0.23
$t_{½}\alpha_2$ (h)	2.13	1.44	2.10	1.74	1.76	1.84	1.84 ± 0.11
B (µg.ml^{-1})	1.10	1.07	0.65	0.90	1.10	0.76	0.93 ± 0.08
β (h^{-1})	0.06	0.07	0.04	0.06	0.06	0.05	0.06 ± 0.003
$t_{½}\beta$ (h)	11.7	10.5	15.4	11.9	11.5	14.4	12.6 ± 0.77
AUC (µg.h.ml^{-1})	28.9	26.2	27.3	25.9	28.3	27.8	27.4 ± 0.48
AUMC (µg. h^2.ml^{-1})	220.7	189.5	184.0	183.4	211.0	198.3	197.8 ± 6.23
Vd_{area} (L.kg^{-1})	1.17	1.16	1.63	1.33	1.18	1.50	1.33 ± 0.08
Vd_B (L.kg^{-1})	1.83	1.87	3.08	2.23	1.82	2.63	2.24 ± 0.21
Vdss (L.kg^{-1})	0.53	0.55	0.49	0.55	0.53	0.52	0.53 ± 0.009
Cl_B (L.kg^{-1}.h^{-1})	0.07	0.08	0.07	0.08	0.07	0.07	0.07 ± 0.001

The values given are mean ± SE of results obtained from 6 animals.

α_1, distribution rate constant first; A_1, zero-time plasma drug concentration intercept of the least square regression line of distribution phase first; $t_{½\ \alpha1}$, distribution half-life first; α_2, distribution rate constant second; A_2, zero-time plasma drug concentration intercept of the least square regression line of disTribution phase second; $t_{½}\alpha_2$, distribution half life second ; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}\beta$, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; $Vd_{B,}$ volume of distribution based on dose; Vd_{SS}, based on average steady state plasma level; Cl_B, total body clearance.

The volume of distribution signifies the drug diffusion into the body tissues (Gilman *et a1* 2011 and Galinsky and Svensson 1995). Volume of distribution (Vd_{area}) and volume of distribution at steady state (Vd_{ss}) were 1.33 ± 0.08 $L.kg^{-1}$ and 0.53 ± 0.009 $L.kg^{-1}$ respectively in buffalo calves following i.v. injection pointing towards better distribution of the parent drug and its metabolite in various body fluids and tissues. The volumes of distribution at steady state (Vd_{ss}) were very less (0.20, 0.30, 0.25, 0.18, 0.30 $L.Kg^{-1}$) in cattle, bull calves, goats, cows, respectively after i.v. administration (Whittem *et al* 1995, Brown *et al* 1996, Courtin *et al* 1997, Tohamy 2008 and Liu *et al* 2010). Moderate binding (40.9 %) of ceftiofur to plasma proteins observed in buffalo calves might resulted in an increase in the volume of distribution in buffalo calves since more free ceftiofur is available for tissue deposition (Tantituvanont *et al* 2009).

In support to longer distribution half life, lower Cl_B (0.07 ± 0.001 $L.kg^{-1}.h^{-1}$) added evidence to slower drug elimination after i.v administration in the present investigation. Similar body clearance (0.066 $L.kg^{-1}.h^{-1}$) was observed in goats after i.v. administration of ceftiofur 2.2 mg $CFAE.kg^{-1}$ B.W. (Courtin *et al* 1997). In contrast, much lesser body clearance (0.032, 0.033, 0.03 and 0.013 $L.kg^{-1}.h^{-1}$) than present study was seen in cattle, cows, bull calves and goats when compared to the present findings (Whittem *et al* 1995, Brown *et al* 1996, Tohamy 2008 and Liu *et al* 2010). In corroboration with smaller Cl_B, the longer elimination half-life ($t_{½}\beta$) was observed after i.v. injection of ceftiofur sodium in buffalo calves (12.6 ± 0.77 h). The elimination half life of ceftiofur sodium was longer in cows (15.3 h) than present study after i.v. administration (Liu *et al* 2010). Shorter $t_{1/2}\beta$ of 7.12, 7.00, 5.33, 4.23, 5.10 h were reported in cattle, bull calves, sheep, goats and cows, respectively

(Whittem *et al* 1995, Brown *et al* 1996, Craigmill *et al* 1997, Courtin *et al* 1997 and Tohamy 2008). The longer elimination seen in buffalo calves might be due to less maturation of organs and/or processes for elimination of ceftiofur and its metabolite when compared to adult animals of different species (Brown *et al* 1996). It has been observed that protein binding extends the biological half-life of ceftiofur due to the protection of the β-lactam ring from cleavages and subsequent reduction of the drug elimination rate by glomerular filtration (Hornish and Kotarski 2002). So, comparatively greater plasma protein binding (40.9 %) in buffalo calves than cow (29.63%) as reported by Tohamy (2008) might be one of the reasons for longer elimination of ceftiofur in buffalo calves.

(ii) Extravascular administration

(a) Intramuscular administration

Pharmacokinetic study of an antibiotic after intramuscular administration is prerequisite for its clinical recommendation. The extent of systemic absorption of a drug directly depends upon its route of administration. Intramuscular route is advised when condition of animal is not critical. Absorption of drug after intramuscular route is good because of better blood supply to muscles than subcutaneous tissues. Extent of systemic absorption varies from drug to drug and even from species to species. For example, intramuscular administration of chloramphenicol produces eratic blood vessels, therefore given only intravenously or orally. Other antimicrobials like erythromycin and cephalothin produces necrosis and myositis upon intramuscular injection (Smith 1984). The pharmacokinetic study of ceftiofur by this route is essential because (i) this route is most commonly used under field conditions especially in large animals like cattle and buffalos, (ii) once apparent equilibrium

between central (blood) and peripheral compartment (tissues) has been attained, i.v. route may not be of much use to increase the transfer of drug from central to peripheral compartment. Keeping this in sight, the kinetic disposition of ceftiofur was also investigated after single intramuscular administration of same dose in same group of animals.

Table 5 presents the plasma concentrations of ceftiofur at different time intervals after single intramuscular administration at the dose rate of 2 $mg.kg^{-1}$ body weight in buffalo calves. Parent drug and its metabolite were detected up to 36 h in the plasma of buffalo calves after dosing of ceftiofur sodium. Semi logarithmic plot of mean plasma ceftiofur and DFC concentration versus time curves following i.m. administration was best fitted into a two compartment open model (Fig 8) and the equation describing model for i.m. route was

$$C_P = Ae^{-\alpha t} + Be^{-\beta t} - A'e^{-Kat}$$

Where, C_P is the plasma concentration of ceftiofur and DFC at time 't'. A', A and B are zero time intercepts of initial absorption, distribution and elimination phase of log concentration time curve, respectively. The Ka, α and β are the rate constants for absorption, distribution and elimination phases, respectively.

The log ceftiofur and DFC concentration versus time data for each animal after i.m. administration was best fitted individually into a two-compartment open model consistent to that reported in calves (Halstead *et al* 1992 and Brown *et al* 1996), goats (Courtin *et al* 1997), sheep (Craigmill *et al* 1997), red deers (Drew *et al* 2004) and cows (Tohamy 2008).

Pharmacokinetic parameters of ceftiofur and desfuroylceftiofur after single intramuscular administration of ceftiofur sodium (2 $mg.kg^{-1}$) in healthy buffalo calves

Table 5: Plasma levels (μg.ml^{-1}) of ceftiofur and desfuroylceftiofur after single intramuscular administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Time (h)	Animals						Mean±SE
	1	2	3	4	5	6	
0.04	1.67	1.34	1.25	0.98	1.73	1.58	1.43 ± 0.12
0.08	3.76	2.94	2.69	2.60	3.24	3.27	3.08 ± 0.18
0.17	4.47	4.76	4.40	4.55	4.14	4.19	4.42 ± 0.09
0.25	5.61	6.90	7.36	7.68	5.29	6.05	6.48 ± 0.40
0.50	4.87	6.17	6.75	5.87	5.74	6.32	5.95 ± 0.26
0.75	4.48	4.97	5.55	5.37	4.75	4.83	4.99 ± 0.16
1.00	3.89	4.42	4.63	4.14	3.93	4.46	4.24 ± 0.12
2.00	2.97	3.13	3.31	3.23	3.02	3.46	3.18 ± 0.08
3.00	2.39	2.26	2.45	2.38	2.33	2.42	2.37 ± 0.03
4.00	1.17	1.26	1.18	1.31	1.25	1.20	1.23 ± 0.02
5.00	0.85	0.93	0.78	1.15	0.97	0.87	0.93 ± 0.05
6.00	0.67	0.71	0.68	0.66	0.85	0.71	0.71 ± 0.03
8.00	0.49	0.53	0.47	0.51	0.52	0.59	0.52 ± 0.02
10.0	0.45	0.40	0.44	0.42	0.44	0.48	0.44 ± 0.01
12.0	0.30	0.35	0.36	0.41	0.40	0.36	0.36 ± 0.02
16.0	0.23	0.25	0.35	0.34	0.30	0.30	0.30 ± 0.02
24.0	0.19	0.17	0.28	0.17	0.22	0.23	0.21 ± 0.02
36.0	0.14	0.11	0.14	0.11	0.11	0.16	0.13 ± 0.01

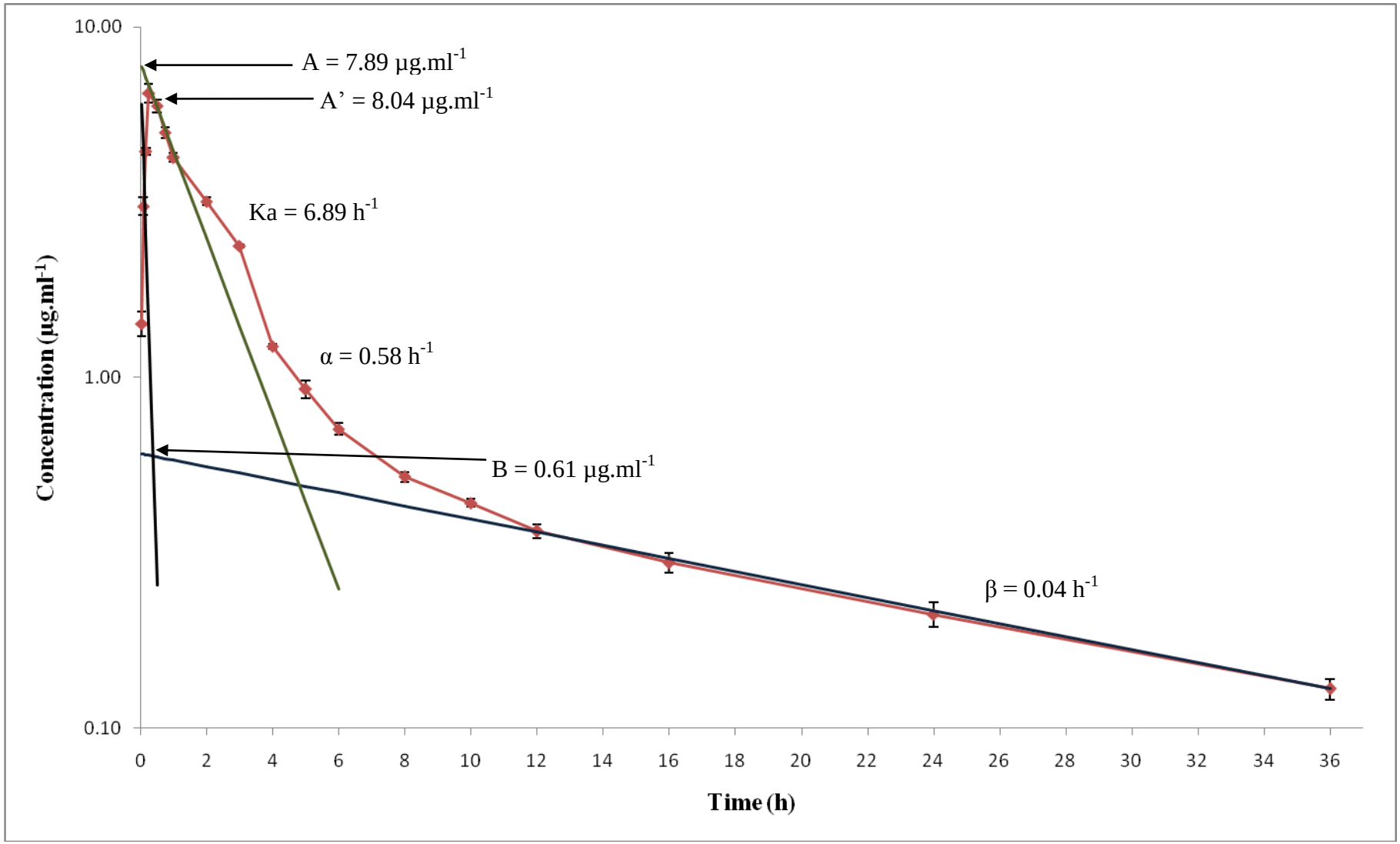


Fig. 8: Semilogarithmic plot of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single intramuscular administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

are presented in table 6. The peak plasma concentration (C_{max}) observed was 6.60 ± 0.35 µg.ml^{-1} at 0.33 ± 0.05 h (T_{max}) after i.m. injection of ceftiofur sodium. Similar C_{max} of 7.13 µg.ml^{-1}, 5.6 µg.ml^{-1} and 7.834 µg.ml^{-1} were reported in sheep and cows, respectively following i.m. administration (Craigmill *et al* 1997 and Tohamy 2008). The C_{max} seen in cattle and 9 month old Holstein bull calves injected CFAE at dose 2.2 mg.kg^{-1} B.W. were 13.9 µg.ml^{-1} and 9.25 µg.ml^{-1}, respectively at t_{max} 0.67–2 h (Brown *et al* 1996, 2000), many folds longer as compared to buffalo calves. However, lower plasma concentration i.e., 4.57 µg.ml^{-1} and 3.98 µg.ml^{-1} were reported in goats and red deers at 1.17 h and 0.54 h administered with ceftiofur via i.m. route (Courtin *et al* 1997 and Drew *et al* 2004). Although there are many variations in the values of maximum plasma concentrations of ceftiofur, the literature on β-lactam antibiotics states that efficacy do not correlate to maximum plasma or tissue concentration but depends on the time length during which concentrations remain above MIC of susceptible pathogen.

Absorption half life ($t_{½}$ka) in the present study was very short (0.11 ± 0.01 h) denoting faster absorption of the drug *via* i.m. route. In comparison to buffalo calves in the present investigation, slow absorption (0.380, 0.27, 0.16 and 0.353 h) was observed in bull calves, non lactating goats, red deer and cows, respectively (Brown *et al* 1996, Courtin *et al* 1997, Drew *et al* 2004 and Tohamy 2008). The fat content and musculature in mature animals might be responsible for slight delay in absorption of the drug besides the long acting formulation. Distribution half life ($t_{1/2}\alpha$) of 1.22 h observed in the current study, denotes better persistence of drug in the tissue and extracellular fluid as compared to shorter distribution half life (0.67 h) seen in red deers (Drew *et al* 2004). Longer distribution half life (2.82 h) observed in bull calves might be because of administration of long acting drug formulation (Brown *et al* 1996).

Table 6: Pharmacokinetic parameters of ceftiofur and desfuroylceftiofur after single intramuscular administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Parameter (Unit)	Animals						Mean ± SE
	1	2	3	4	5	6	
Ka (h^{-1})	7.91	8.58	5.14	6.32	8.30	5.11	6.89 ± 0.64
A ($\mu g.ml^{-1}$)	6.19	8.49	10.11	10.08	5.67	7.68	8.04 ± 0.77
$t_{½}$ka (h)	0.09	0.08	0.13	0.11	0.08	0.14	0.11 ± 0.01
α (h^{-1})	0.50	0.54	0.67	0.65	0.51	0.57	0.58 ± 0.03
A ($\mu g.ml^{-1}$)	6.77	7.44	9.74	9.00	6.10	8.31	7.89 ± 0.56
$t_{½}$α (h)	1.38	1.28	1.03	1.06	1.35	1.21	1.22 ± 0.06
B ($\mu g.ml^{-1}$)	0.40	0.58	0.63	0.78	0.73	0.53	0.61 ± 0.06
β (h^{-1})	0.03	0.05	0.04	0.06	0.05	0.03	0.04 ± 0.004
$t_{½}$β (h)	22.97	14.55	17.64	12.43	13.50	20.90	17.0 ±1.74
AUC ($\mu g.h.ml^{-1}$)	26.20	25.06	28.77	25.94	25.50	29.45	26.8 ± 0.75
AUMC ($\mu g.\ h^2.ml^{-1}$)	177.90	157.40	207.06	170.10	178.63	210.41	183.6 ± 8.55
Vd_{area} ($L.kg^{-1}$)	2.53	1.68	1.77	1.38	1.53	2.05	1.82 ± 0.17
F (%)	46.26	69.16	91.87	95.84	76.95	73.26	75.6 ± 7.27
Vd_B ($L.kg^{-1}$)	5.01	3.47	3.18	2.55	2.74	3.80	3.46 ± 0.36
Cl_B ($L.kg^{-1}.h^{-1}$)	0.08	0.08	0.07	0.08	0.08	0.07	0.08 ± 0.002
C_{max} ($\mu g.ml^{-1}$)	5.61	6.90	7.36	7.68	5.74	6.32	6.60 ± 0.35
T_{max} (h)	0.25	0.25	0.25	0.25	0.50	0.50	0.33 ± 0.05

The values given are mean ± SE of results obtained from 6 animals.

Ka, absorption rate constant; A', zero-time plasma drug concentration intercept of the least square regression line of absorption phase; $t_{1/2ka}$, absorption half-life; α, distribution rate constant; A, zero-time plasma drug concentration intercept of the least square regression line of disribution phase $t_{½}$α, distribution half life; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}$β, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; F – Bioavailability; Vd_B, volume of distribution based on dose; Cl_B , total body clearance; C_{max}, plasma drug concentration; T_{max}, time at which C_{max} is attained

The value of area under curve (AUC) in the present study (26.8 ± 0.75µg.h. ml^{-1}) was similar to observed by Craigmill *et al* (1997) in sheep (33.7 µg.h.ml^{-1}), but more than reported (14.43 µg.h.ml^{-1}) in red deer (Drew *et al* 2004) after i.m. injection. However, much greater AUCs (77.3, 124.1, 108 and 72.098 µg.h.ml^{-1}) were observed in bull calves, goats, cattle and cows, respectively (Brown *et al* 1996, 2000, Courtin *et al* 1997 and Tohamy 2008). This may be due to difference in the amount of body fluids among different species at various ages along with administration of long acting CFAE preparation of administered. The volume of distribution is an accurate indicator of the drug diffusion into the body tissues. Volume of distribution (Vd_{area}) was 1.82 ± 0.17 $L.kg^{-1}$ in buffalo calves which indicates better distribution of the antimicrobial in various body fluids and tissues. Similar distribution (Vd_{ss} - 1.67 $L.kg^{-1}$) was seen in red deer following i.m. administration (Drew *et al* 2004). Moderate binding (40.9 %) of ceftiofur to plamsa proteins as observed in the present investigation might be responsible for an increase in the volume of distribution in buffalo calves since more free parent drug along with its active metabolite is available for tissue deposition (Tantituvanont *et al* 2009). In comparison to the current study, lesser volume of distribution **(**Vd_{ss} = 0.37 $L.kg^{-1}$) was reported in sheep (Craigmill *et al* 1997).

In support to longer $t_{1/2}\alpha$, low value of Cl_B (0.08 ± 0.002 $L.kg^{-1}.h^{-1}$) added confirmation to slower drug elimination in the present investigation. Body clearance of ceftiofur in red deers (0.15 $L.kg^{-1}.h^{-1}$) was almost double than reported in present study (Drew *et al* 2004). In confirmation with smaller Cl_B, the longer elimination half-life ($t_{½}\beta$) was observed after i.m. injection of ceftiofur sodium in buffalo calves (17.0 ±1.74 h). Shorter $t_{1/2}\beta$ of 11.2, 2.6, 12.8 and 5.03 h were reported in bull calves, goats, cattle, and cows, respectively (Brown *et al* 1996, 2000, Courtin *et al* 1997 and

Tohamy 2008). The longer elimination seen in buffalo calves might be due to less maturation of organs and/or processes for elimination of ceftiofur and its metabolite as compared to adult animals of different species (Brown *et al* 1996). Another reason for slow elimination might be rapid hydrolysis of ceftiofur after i.m. injection to DFC which is highly protein bound and having longer elimination half-life than parent drug (Jaglan *et al* 1994 and Salmon *et al* 1996). On the contrary, longer elimination (23.0 h) seen in red deers (Drew *et al* 2004) compared to present investigation may be due to variation in the enzymatically catalysed metabolism and renal excretion of ceftiofur, including active renal secretion via organic acid transporters, as well as glomerular filtration in these species (Brown *et al* 1996).

The literature on i.m. administration of ceftiofur states that bioavailability (F) of the drug in cattle and buffalo calves were 89.8 and 99.7 %, respectively (El-Gendy *et al* 2007). Bioavailability in cows (81.9 %) was similar to that observed in present study (75.6 ± 7.27 %). Thus, bioavailability of ceftiofur sodium observed in the present study reflects fair absorption of the drug into systemic circulation from the site of i.m. injection in buffalo calves.

(b) Subcutaneous administration

Injection of drug into a subcutaneous site is often used when sustained effect is desired. It can be used for drugs that are nonirritating to tissue, as severe pain, necrosis and sloughing of the skin may occur. The rate of absorption of a drug following s.c. administration is often adequately constant and slow so as to produce a sustained effect. To avoid muscle soreness following i.m. administration, some practitioners have administered ceftiofur through s.c. route (Salmon *et al* 1996, Samitz *et al* 1996 and Slovis *et al* 2006). Subcutaneous administration of drug may be

chosen in the conditions when rapid absorption, good availability of drugs along with ease of administration is needed. Keeping this in sight, the pharmacokinetic disposition of ceftiofur was also studied in buffalo calves, after its single subcutaneous administration at the same dose rate in the same group of animals.

The plasma concentration of ceftiofur at different time points after single subcutaneous administration at the dose rate of 2 $mg.kg^{-1}$ body weight in buffalo calves are depicted in Table 7. Ceftiofur and DFC were detected up to 48 h in the plasma of buffalo calves after administration of ceftiofur sodium. Semi logarithmic plot of mean plasma ceftiofur and DFC concentration versus time curves after s.c. administration was best fitted into a two compartment open model (Fig 9) and the equation describing model for s.c. route was

$C_P = Ae^{-\alpha t} + Be^{-\beta t} – A'e^{-Kat}$

Where, C_P is concentration of ceftiofur and DFC in plasma at time 't'. A', A and B are zero time intercepts of initial absorption, distribution and elimination phase of log concentration time curve, respectively. The Ka, α and β are the rate constants for absorption, distribution and elimination phases, respectively.

The dose of ceftiofur sodium in the present study was comparable to that used in previous studies in cattle after subcutaneous dosing (Brown *et al* 1996, 2000 and Okker *et al* 2002). The various pharmacokinetic parameters for ceftiofur and its metabolite DFC were calculated and presented in table 8. The peak plasma concentration (C_{max}) observed was $2.99 \pm 0.05 \mu g.ml^{-1}$ at 0.92 ± 0.05 h (T_{max}) after s.c. injection of ceftiofur. Maximum plasma concentration (C_{max}) as similar to that in lactating dairy cows after s.c. injection of ceftiofur hydrochloride at 1 $mg.kg^{-1}$ B.W. (Okker *et al* 2002) at almost double t_{max} observed in the present study. However, C_{max} of 13.6 $\mu g.ml^{-1}$ was observed in another study in cattle administered with 2.2 mg

Table 7: Plasma levels (μg.ml^{-1}) of ceftiofur and desfuroylceftiofur after single subcutaneous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Time (h)	Animals						Mean± SE
	1	2	3	4	5	6	
0.04	0.17	0.26	0.18	0.24	0.19	0.12	0.19 ± 0.02
0.08	0.28	0.56	0.40	0.49	0.34	0.37	0.41 ± 0.04
0.17	0.77	1.23	1.09	0.96	0.78	0.78	0.93 ± 0.08
0.25	1.44	1.66	1.58	1.78	1.84	1.36	1.61 ± 0.08
0.50	1.96	2.04	2.31	2.42	2.19	2.59	2.25 ± 0.10
0.75	2.55	3.18	2.78	2.96	2.47	2.96	2.81 ± 0.11
1.00	2.86	3.12	3.00	2.86	2.86	3.07	2.96 ± 0.05
2.00	2.59	2.56	2.76	2.55	2.61	3.06	2.69 ± 0.08
3.00	2.14	2.24	2.56	2.30	2.22	2.81	2.38 ± 0.10
4.00	2.06	1.81	2.39	1.96	1.76	2.55	2.09 ± 0.13
5.00	1.86	1.68	1.93	1.70	1.58	2.15	1.82 ± 0.08
6.00	1.54	1.53	1.80	1.48	1.36	1.76	1.58 ± 0.07
8.00	1.29	1.28	1.29	1.24	1.15	1.37	1.27 ± 0.03
10.0	1.10	1.05	1.09	1.01	0.93	1.20	1.06 ± 0.04
12.0	0.82	0.81	0.90	0.79	0.77	0.94	0.84 ± 0.03
16.0	0.55	0.57	0.58	0.51	0.51	0.64	0.56 ± 0.02
24.0	0.41	0.40	0.39	0.38	0.35	0.42	0.39 ± 0.01
36.0	0.24	0.23	0.25	0.25	0.21	0.25	0.24 ± 0.01
48.0	0.13	0.15	0.16	0.16	0.13	0.13	0.14 ± 0.01

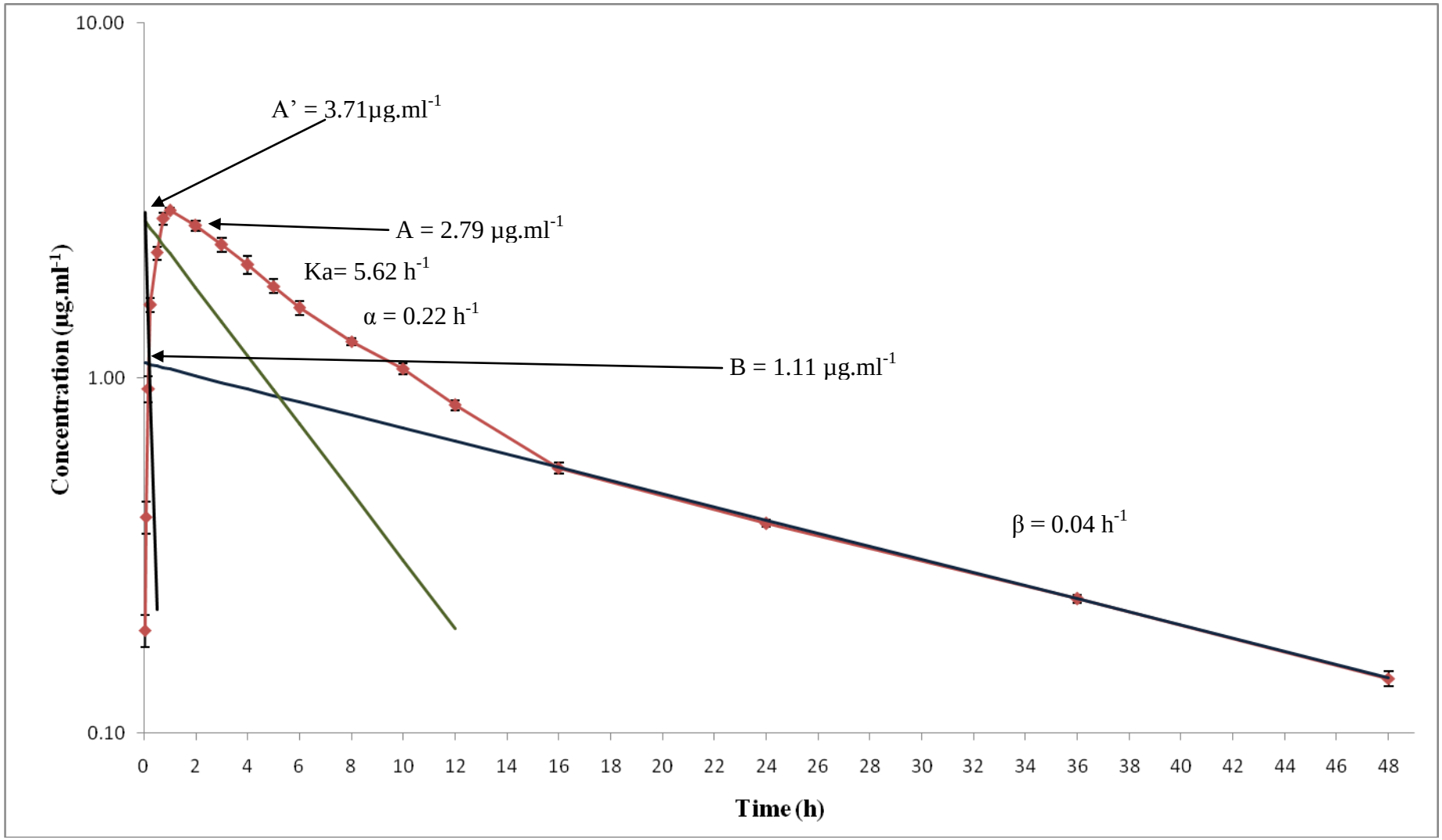


Fig. 9: Semilogarithmic plot of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single subcutaneous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Table 8: Pharmacokinetic parameters of ceftiofur and desfuroylceftiofur after single subcutaneous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy buffalo calves.

Parameter (Unit)	Animals						Mean ± SE
	1	2	3	4	5	6	
Ka (h^{-1})	4.55	6.67	3.53	8.02	6.71	4.26	5.62 ± 0.72
A ($\mu g.ml^{-1}$)	3.16	3.05	3.68	4.74	3.04	4.61	3.71 ± 0.32
$t_{½}$ka (h)	0.15	0.10	0.20	0.09	0.10	0.16	0.13 ± 0.02
α (h^{-1})	0.22	0.21	0.22	0.21	0.21	0.25	0.22 ± 0.007
A ($\mu g.ml^{-1}$)	2.55	2.39	3.27	2.76	2.30	3.50	2.79 ± 0.20
$t_{½}$α (h)	3.11	3.25	3.20	3.31	3.29	2.73	3.15 ± 0.09
B ($\mu g.ml^{-1}$)	1.18	1.09	1.05	0.90	0.98	1.44	1.11 ± 0.08
β (h^{-1})	0.05	0.04	0.04	0.04	0.04	0.05	0.04 ± 0.002
$t_{½}$β (h)	15.18	16.58	17.49	19.44	16.50	13.78	16.5 ± 0.79
AUC ($\mu g.h.ml^{-1}$)	35.22	35.97	37.29	36.51	33.15	39.04	36.2 ± 0.81
AUMC ($\mu g.\ h^2.ml^{-1}$)	549.55	592.13	562.32	646.21	531.61	564.95	574.5 ± 16.5
Vd_{area} ($L.kg^{-1}$)	1.24	1.33	1.35	1.54	1.44	1.02	1.32 ±0.07
F (%)	67.32	61.79	82.17	58.26	56.54	103.58	71.6 ± 7.43
Vd_B ($L.kg^{-1}$)	1.69	1.84	1.90	2.22	2.04	1.39	1.85 ± 0.12
Cl_B ($L.kg^{-1}.h^{-1}$)	0.06	0.06	0.05	0.05	0.06	0.05	0.06 ± 0.001
C_{max} ($\mu g.ml^{-1}$)	2.86	3.18	3.00	2.96	2.86	3.07	2.99 ± 0.05
T_{max} (h)	1.00	0.75	1.00	0.75	1.00	1.00	0.92 ± 0.05

The values given are mean ± SE of results obtained from 6 animals.

Ka, absorption rate constant; A', zero-time plasma drug concentration intercept of the least square regression line of absorption phase; $t_{1/2ka}$, absorption half-life; α, distribution rate constant; A, zero-time plasma drug concentration intercept of the least square regression line of disribution phase $t_{½}$α, distribution half life; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}$β, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; Vd_B, volume of distribution based on dose; Cl_B, total body clearance; C_{max}, peak drug concentration; T_{max}, time to C_{max}.

ceftiofur equivalents/kg as ceftiofur sodium subcutaneously which was much higher than present investigation (Brown *et al* 2000).

Absorption half life ($t_{½}ka$) in the present study was (0.13 ± 0.02 h) very short than reported in ruminant species indicating faster absorption of the drug after s.c. injection. The absorption half life was three times longer (0.38 h) in 9 month old cow calves after i.m. administration at the dose rate of 2.2 $mg.kg^{-1}$ B.W. (Brown *et al* 1996) when compared to present study. The fat content in mature cattle may be one of the reasons for slow absorption of the drug in addition to the long acting formulation. Two times $t_{½}ka$ (0.27 h) observed in another pharmacokinetic study in goats might be due to administration of long acting crystalline free acid equivalents (Courtin *et al* 1997). Distribution half life ($t_{½}\alpha$) of 3.15 ± 0.09 h was observed in the present study indicates better persistence of drug in the tissue and extracellular fluid. Consistent to the present findings, distribution half life of 2.82 h was observed in the i.m. study conducted by Brown and co workers (1996).

The value of area under curve (AUC) was 36.2 ± 0.81 $\mu g.h.ml^{-1}$ shows longer plasma exposure of the drug in the body adding to its antimicrobial activity against susceptible microorganisms. However, it was lesser than that observed in cows following s.c. administration of ceftiofur hydrochloride (Okker *et al* 2002). In comparison to the present study, AUC seen in 9 month old cow calves was more than two times (77.3 $\mu g.h.ml^{-1}$) higher, which might be due to difference in the body fluids at the early age (Brown *et al* 1996). In addition, the values seen in cattle after i.m. and s.c. injection of ceftiofur sodium at the dose rate of 2.2 CFAE/kg B.W. were 108 and 105 $\mu g.h.ml^{-1}$, respectively (Brown *et al* 2000). Possible reason behind this difference can be age and species variation of the experimental animals. AUC similar to the present study was observed in goats administered with ceftiofur sodium at 2.2 $mg.kg^{-1}$ B.W. (Courtin *et al* 1997). Much greater values of the AUC observed in non lactating

goats (159.35 µg.h.ml^{-1}) might be due to long acting CFAE preparation of the drug given at the dose rate more than three times used in the present investigation (Dore *et al* 2011).

Volume of distribution (Vd_{area} = 1.32 ± 0.07 L.kg^{-1}) in the present study indicated better distribution of the antimicrobial in various body fluids and tissues. Larger Vd_{area} reported in the non lactating goats (Dore *et al* 2011) might be due to long acting formulation, dose and species variation. The volume of distribution is a precise indicator of the drug diffusion into the body tissues (Gilman *et a1* 2011 and Galinsky and Svensson 1995). The greater Vd_{area} after s.c. administration points towards rapid and higher penetration of the drug to the extra-vascular tissues in buffalo calves.

The longer pesistance of ceftiofur and DFC as observed through better distribution half life was further confirmed by lower Cl_B (0.06 ± 0.001 L.kg^{-1}.h^{-1}). In support to smaller body clearance, the longer (16.5 ± 0.79 h) elimination half-life ($t_{½}\beta$) was observed after ceftiofur administration in buffalo calves. The $t_{1/2}\beta$ in the study was 1.5 times longer than seen in 9 month old cattle calves (11.2 h) after i.m. injection of ceftiofur sodium (Brown *et al* 1996). The difference may be due to species variation, different route and drug preparations used in the study. In comparison to present study, $t_{½}\beta$ (36.9 h) was more than two times greater in goats after s.c. administration which might be due to long acting CFAE preparations of ceftiofur used in that pharmacokinetic study (Dore *et al* 2011). The longer elimination half life (16.5 h) along with smaller Cl_B indicates slow excretion of the drug from the animal body. Elimination half life (16.5 h) in the present study was 2.5 times (Brown *et al* 2000) than in cattle (9.84 h) which could be due to maturation of the organs and/or processes for elimination of ceftiofur and it's desfuroylceftiofur

metabolite in cattle than buffalo calves (Brown *et al* 1996). The least $t_{½}\beta$ (2.6 h) in goats following i.m. dosing of 2.2 mg CFAE/kg (Courtin *et al* 1997) might be due to variation in the age, species and formulation used.

Thus bioavailability of ceftiofur sodium after s.c. administration in present study (71.6 ± 7.43 %) reflects fair absorption of the drug into systemic circulation similar to that observed after i,m (75.6 ± 7.27 %) dosing in buffalo calves. This indicates that ceftiofur sodium can be administered by this route for the treatments of less severe infection when frequent administration of ceftiofur is not possible.

The physiological changes that occur in mammals from birth to maturity may affect the pharmacokinetics of antimicrobials. Body composition during maturation may affect distribution to various tissues and body fluids. Maturation of organs of metabolism and/ excretion like liver and kidneys affects respective phases of biotransformation (Brown *et al* 1996). Variation in the elimination phase from previous studies might be because of duration of sample collected post administration and limit of detection (LOD) for different assay methodologies used for quantification of ceftiofur and DFC. These two are very crucial for accurate estimation of elimination rate constant in addition to difference in protein binding across species (Whittem *et al* 1995). The elimination rate constants are very much important for accurate estimation of subsequent distribution and absorption rate constants (in case of extravascular administration) for the least square regression analysis (feathering technique). The rate constants are further used in calculation of various pharmacokinetic parameters using different equations.

2. *In vitro* plasma protein binding

Protein bound fraction of an antimicrobial does not have any antibacterial activity and only free fraction of the drug possesses this activity. The extent of protein

binding can also directly affect the therapeutic efficacy of drug (Craig and Kunin 1976). The unbound fraction of drug is also desired for distribution and clearance (Wise *et al* 1980 and Yamada *et al* 1981). The efficacy of cephalosporins has direct correlation with the time length for which its unbound concentration remains above MIC as percent of dosing interval (Andes and Craig 2002). *In vitro* antibacterial activity of an antibiotic is directly proportional to the plasma protein concentration and fraction antibiotic bound to plasma proteins (Petersion *et al* 1975). So for evaluation of the potential efficacy of ceftiofur in healthy buffalo calves, its plasma proteins binding should be known.

Earlier studies postulated that significant increase in the serum concentration of ceftiofur with multiple doses without increase in the elimination half life may be due to saturation of protein binding sites (Halstead *et al* 1992). Later, it was found that the binding of ceftiofur to serum proteins was 29.63% in healthy cow calves (Tohamy 2008). The extent of plasma protein binding varied from 34.6 - 47.1% in healthy buffalo calves with overall mean of 40.9 ± 1.49 % (table 9). The difference might be due to variation in the species tested and assay method followed in these studies. The extent of protein binding reported in goats (46.6%) was more than reported in buffalo calves (Singh 2014). The concentrations selected for protein binding study was in the range of 1 to 10 $\mu g.ml^{-1}$. Binding of ceftiofur to plasma proteins was not concentration dependent in buffalo calves. Moderate plasma protein binding resulted in an increase in the volume of distribution of ceftiofur and its metabolite in buffalo calves since more free drug is available for tissue deposition (Tantituvanont *et al* 2009).

Binding pattern of ceftiofur to plasma proteins was not concentration dependent in buffalo. The binding capacity of drug to plasma proteins (β_i) and

dissociation rate constant of protein drug complex (K_β) of the drug-protein complex quantitatively describe the drug protein interaction. The (β_i) and (K_β) were 2.54 ± 0.12 x 10^{-5} mole/g and 2.00 ± 0.08 x 10^{-6} mole, respectively. The higher value of β_i than K_β indicated that binding of ceftiofur to plasma proteins was relatively faster than dissociation of protein drug complex in buffalo calves.

Table 9. Kinetic constants of *in vitro* ceftiofur binding to plasma proteins of healthy buffalo calves (n=6)

Expt. No.	Protein binding (%) Ceftiofur concentration (µ g/ml)					β_i (10^{-5} mole/g)*	K_β (10^{-6}mole)**
	1	3	4	5	10		
1	37.5	47.1	41.7	36.4	37.1	2.3	1.88
2	40.1	42.7	44.9	34.6	40.7	2.63	1.97
3	38.8	45.8	43.9	40.6	41.6	2.69	2.15
Mean ± SEM	38.8 ± 0.75	45.2 ± 1.31	43.5 ± 0.95	37.2 ± 1.78	39.8 ± 1.37	2.54 ± 0.12	2.00 ± 0.08

* Association rate constant; ** Dissociation rate constant
Overall (mean ± SEM) binding = 40.9 ± 1.49 %

B. Pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in febrile buffalo calves

i) Induction of fever model

In the present study *E. coli* endotoxin at the dose rate of 1µg.kg^{-1} body weight by i.v. route (Sharma and Ul-Haq 2012a) was used to induce fever in buffalo calves. The effect of *E. coli* endotoxin on body temperature of buffalo calves was recorded and is presented in table 10. There was great animal to animal variation in the body temperature during induction of fever in buffalo calves. In some of the buffalo calves there was significant rise in body temperature within 2 - 2.5 h of administration of *E.*

coli lipopoysaccharide (LPS) however, in some cases, the dose of endotoxins was repeated so as to get an optimum increase in body temperature (1-2 ^{0}F) within 2 h of first dose. As the drug was detected up to 36 h in the plasma of healthy calves, the dose of LPS was repeated again when necessary so to maintain increased rectal temperature in feverish buffalo calves. After the i.v. injection of LPS, in addition to rise in body temperature, some other symptoms of pyrexia like increased respiration, decreased water and feed intake, dullness, loose motion, dryness of mouth and muzzles were also observed in buffalo calves.

Fever is a usual consequence of many infectious diseases. The bacterial endotoxin induces fever through production of endogenous pyrogens i.e. interlukins and TNF (Gordon *et al* 2005). Febrile state has been reported to alter the pharmacokinetics and thus dosage regimen of many cephalosporins in cattle and buffalo calves (Chaudhary *et al* 1999, 2001, Dardi *et al* 2005, Pawar and Sharma 2008, Rajput *et al* 2008 and Joshi and Sharma 2009). Pharmacokinetic of ceftiofur sodium has also been shown to be altered in feverish cows (Tohamy 2008). However, no study has been carried out in buffalo species. Therefore to formulate the dosage regimen of ceftiofur in febrile state, it was thought to carry out pharmacokinetic study in buffalo calves.

ii) Plasma levels and pharmacokinetics

In animals, the indiscriminate use of antibiotics is a global concern due to their impact on the environment, human health and for development of antibiotic resistance (Vilos *et al* 2012). Therefore, for effective treatment and reduction of further complications, an appropriate change in dosage regimen is necessary (Tantituvanont *et al* 2009). Rational antibiotic therapy requires dosage regimens to be optimized for its clinical efficacy as well as to minimize the selection and spread of resistant

Table 10: Body temperature (^{0}F) of buffalo calves in which fever was induced with single or repeated injection of *E.coli* endotoxin (1 µg.kg^{-1} B.W.) to study the pharmacokinetics and *in vitro* plasma protein binding of ceftiofur.

Time after endotoxin (LPS) administration (h)	Animal number						Mean ± SE
	1	2	3	4	5	6	
0	100.1	99.5	98.3	99.4	100.2	98.7	99.37 ± 0.31
0.5	100.1	101.2	98.4	99.9	100.1	99.0	99.78 ± 0.40
1	100.0	101.8	98.5	100.6	100.3	99.3	100.08 ± 0.46
1.5	100.1	102.4	98.7	100.9	100.5	99.7	100.38 ± 0.51
2	100.2[a]	102.8[b]	99.7[a]	101.2[b]	100.5[a]	100.0	100.73 ± 0.46
2.5	101.0	103.0	100.3[b]	101.3	100.9	100.4[b]	101.15 ± 0.40
3	101.2	102.8	100.5	101.3	101.1	101.6	101.42 ± 0.31
3.5	101.6	102.7	100.7	101.5	101.6	101.7	101.63 ± 0.26
4	102.0[b]	102.8	100.8	101.4	102.0[b]	101.8	101.80 ± 0.27
5	102.5	102.5	100.9	101.4	102.1	101.8	101.87 ± 0.26
8	102.8	102.4	100.7	101.1	102.1	101.7	101.80 ± 0.32
12	102.6	102.1[a]	100.5	100.8[a]	102.1	101.5	101.60 ± 0.33
24	102.2[a]	102.9	100.1[a]	101.7	101.5[a]	101.0[a]	101.57 ± 0.39
36	102.5	102.6	100.6	101.3	102.2	101.8	101.83 ± 0.31
48	101.5	102.2	100.3	101.1	102.0	101.4	101.42 ± 0.28

[a]Time of repetition of endotoxin injection.
[b]Time administration of ceftiofur

pathogens. Pharmacokinetic studies provide a foundation for determination of satisfactory dosage regimen which is relevant, when they are carried out in the species in which the antimicrobials are to be used clinically (Sharma and Ul Haq 2012a).

The studies in cattle and buffalo species have underlined the differences in the pharmacokinetics of cephalosporin antibiotic (Joshi and Sharma 2009). It was also recommended not to extrapolate the data of one species to other species of animal without conducting the detailed pharmacokinetic study (Sharma and Srivastava 2006). The plasma concentrations of ceftiofur at different time points after single intravenous administration in febile buffalo calves are presented in table 11. Comparative plots of mean plasma concentration-time profile of ceftiofur and its metabolite following single intravenous administration of ceftiofur sodium (2 $mg.kg^{-1}$) in healthy and febrile buffalo calves are depicted in figure 10. Parent drug and DFC were detected up to 24 h post treatment in the plasma of febrile animal as compared to 36 h post dosing in healthy animals. It was observed from plots that at most of the times plasma levels of ceftiofur and DFC were less in febrile animals as compared to healthy. The logarithm of mean plasma ceftiofur and DFC concentration versus time curves in feverish animals (Fig 11) were best fitted into a three compartment open model similar to healthy one. The data in febrile buffalo calves was be best described by the equation

$$C_P = A_1e^{-\alpha 1t} + A_2e^{-\alpha 2t} + Be^{-\beta t}$$

Where, CP is ceftiofur and DFC concentration in plasma at time 't'. A1, A2, are zero time intercepts of distribution phases of log concentration time curve and B is zero time intercepts of elimination phase of log concentration time curve, respectively. The α_1, α_2 are the distribution rate constants and and β is elimination rate constant, respectively. Ceftiofur and DFC was less in febrile animals as compared to healthy.

Table 11: Plasma levels (µg.ml^{-1}) of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in febrile buffalo calves.

Time (h)	Animals						Mean± SE
	1	2	3	4	5	6	
0.04	10.6	10.0	9.52	8.63	8.46	7.70	9.16 ± 0.45
0.08	8.31	8.83	7.66	6.06	6.13	5.58	7.10 ± 0.55
0.17	7.65	8.22	6.08	4.41	5.57	3.85	5.96 ± 0.71
0.25	5.39	6.70	4.62	4.09	4.55	3.26	4.77 ± 0.48
0.50	3.43	5.22	3.04	3.32	2.59	2.76	3.39 ± 0.39
0.75	2.99	3.77	1.80	2.07	1.90	1.82	2.39 ± 0.33
1.00	2.23	3.10	1.58	1.37	1.41	1.16	1.81 ± 0.30
2.00	1.43	1.81	0.82	0.83	0.69	0.74	1.05 ± 0.19
3.00	1.17	1.21	0.71	0.63	0.58	0.54	0.81 ± 0.12
4.00	0.89	1.10	0.55	0.42	0.40	0.45	0.63 ± 0.12
5.00	0.82	0.88	0.48	0.40	0.37	0.38	0.56 ± 0.09
6.00	0.71	0.78	0.48	0.36	0.26	0.33	0.48 ± 0.09
8.00	0.70	0.67	0.41	0.32	0.20	0.30	0.43 ± 0.08
10.0	0.54	0.53	0.33	0.20	0.16	0.25	0.33 ± 0.07
12.0	0.35	0.40	0.18	0.17	0.13	0.18	0.25 ± 0.05
16.0	0.25	0.26	0.18	0.12	0.12	0.14	0.18 ± 0.03
24.0	0.22	0.13	0.12	0.09	0.08	0.11	0.13 ± 0.02

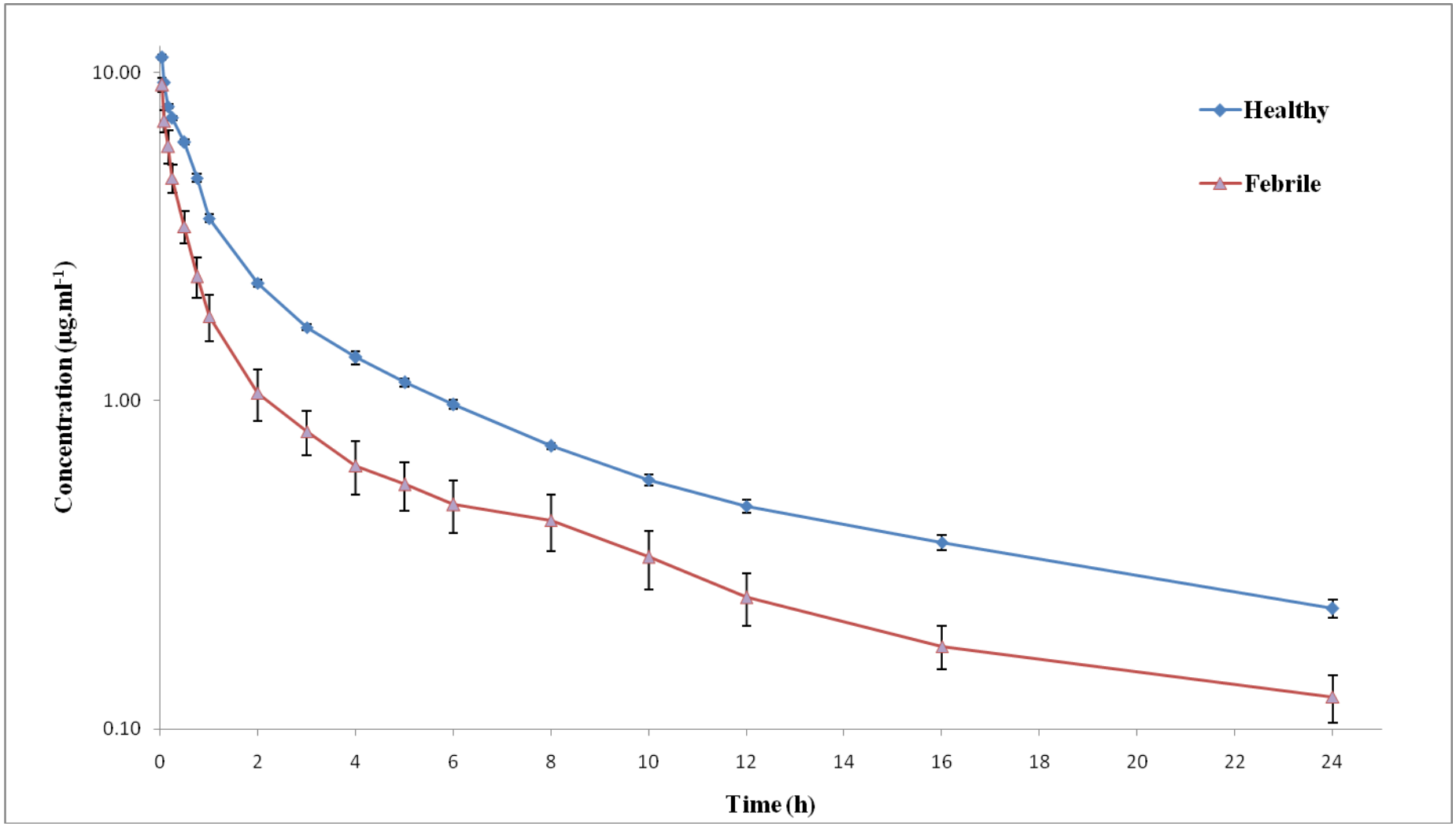


Fig. 10: Comparative plots of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy and febrile buffalo calves.

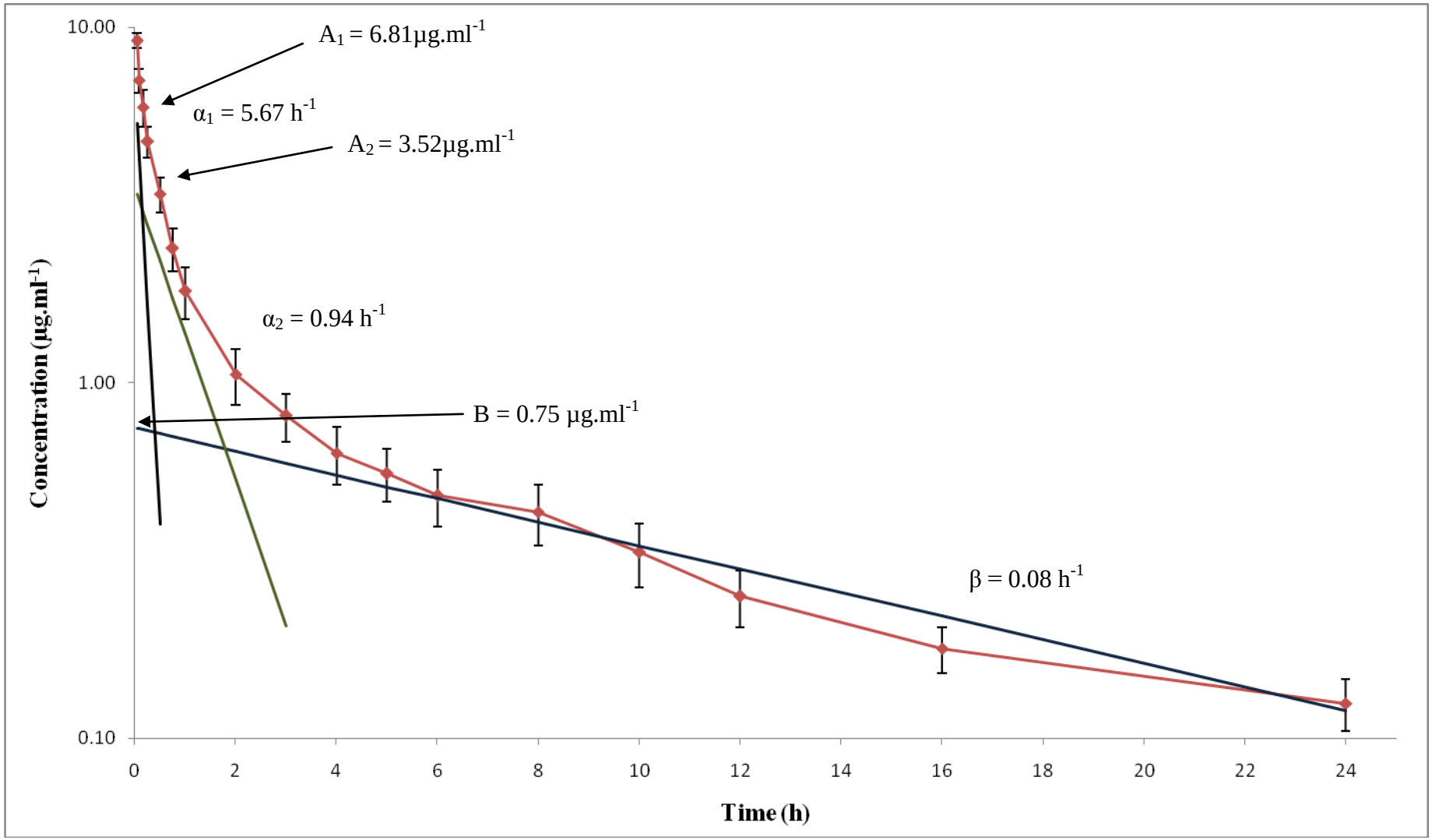


Fig. 11: Semilogarithmic plot of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in febrile buffalo calves.

The pharmacokinetic parameters for ceftiofur and its metabolite DFC following intravenous administrations of ceftiofur sodium in feverish buffalo calves are presented in table 12. Table 13 represents comparative pharmacokinetics of ceftiofur and desfuroylceftiofur in healthy and febrile buffalo calves. The significantly higher values of the distribution rate constant α_2 (0.94 ± 0.07 h^{-1}) in febrile animals indicated that ceftiofur was rapidly distributed into various body fluids and tissue compartments than healthy animals ($\alpha_2 = 0.38 \pm 0.02$ h^{-1}). Similar increase in distribution rate constant was also reported in feverish cows after i.v. administration of ceftiofur sodium (Tohamy 2008). Distribution half life indicated less presence of parent drug and its metabolite in the tissue and extracellular fluid in febrile buffalo calves. Similar decrease in distribution half life in febrile cows was observed by Tohamy (2008). Alterations in the permeability of biological membrane barrier and/or tissue and plasma pH during fever might be responsible for distribution pattern of ceftiofur in fever induced buffalo calves (Pawar and Sharma 2008).

The value of area under curve (AUC) represents the plasma exposure of drug in the body and thus its antimicrobial activity against susceptible pathogens. Significantly decreased AUC (14.0 ± 1.93 $\mu g.h.ml^{-1}$) in feverish buffalo calves denoted that less area of the body was covered by ceftiofur and DFC concentration along with reduced persistence of the drug compared to healthy calves ($AUC=27.4 \pm 0.48$ $\mu g.h.ml^{-1}$). Similarly, decrease in AUC (47.563 $\mu g.\ ml^{-1}.h^{-1}$) in feverish cows than healthy (87.996 $\mu g.ml^{-1}.h^{-1}$) was reported (Tohamy 2008).

The volume of distribution signifies the drug diffusion into the body tissues (Gilman *et a1* 2011 and Galinsky and Svensson 1995). The volume of distribution at steady state (Vd_{ss}) is the constant that expresses the amount of the drug in the body at steady state as a proportion of the corresponding expected plasma

Table 12: Pharmacokinetic parameters of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in febrile buffalo calves.

Parameter (Unit)	Animals						Mean ±SE
	1	2	3	4	5	6	
α_1 (h^{-1})	3.26	2.02	5.11	10.13	4.46	9.06	5.67 ± 1.32
A_1 ($\mu g.ml^{-1}$)	7.54	5.23	7.25	7.40	6.11	7.32	6.81 ± 0.38
$t_{½}$ α_1 (h)	0.21	0.34	0.14	0.07	0.16	0.08	0.17 ± 0.04
α_2 (h^{-1})	0.84	0.99	1.23	0.99	0.74	0.87	0.94 ± 0.07
A_2 ($\mu g.ml^{-1}$)	3.53	5.17	3.58	3.52	2.80	2.54	3.52 ± 0.37
$t_{½}\alpha_2$ (h)	0.82	0.70	0.56	0.70	0.94	0.80	0.75 ± 0.05
B ($\mu g.ml^{-1}$)	1.04	1.43	0.72	0.50	0.32	0.46	0.75 ± 0.17
β (h^{-1})	0.07	0.10	0.08	0.08	0.06	0.07	0.08 ± 0.01
$t_{½}\beta$ (h)	9.63	6.79	8.90	8.87	11.3	10.4	9.32 ± 0.63
AUC ($\mu g.h.ml^{-1}$)	19.9	20.1	12.7	11.1	9.79	10.6	14.0 ± 1.93
AUMC ($\mu g. h^2.ml^{-1}$)	113.0	101.8	65.2	50.6	42.2	54.2	71.2 ± 11.9
Vd_{area} ($L.kg^{-1}$)	1.40	0.98	2.02	2.30	3.34	2.83	2.15 ± 0.36
Vd_B ($L.kg^{-1}$)	1.92	1.39	2.78	3.99	6.33	4.30	3.46 ± 0.74
Vdss ($L.kg^{-1}$)	0.57	0.50	0.81	0.82	0.88	0.97	0.76 ± 0.07
Cl_B ($L.kg^{-1}.h^{-1}$)	0.10	0.10	0.16	0.18	0.20	0.19	0.16 ± 0.02

The values given are mean ± SE of results obtained from 6 animals.

α_1, distribution rate constant first; A_1, zero-time plasma drug concentration intercept of the least square regression line of distribution phase first; $t_{½}$ α_1, distribution half-life first; α_2, distribution rate constant second; A_2, zero-time plasma drug concentration intercept of the least square regression line of disribution phase second; $t_{½}\alpha_2$, distribution half life second ; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}\beta$, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; $Vd_{B,}$ volume of distribution based on dose; Vd_{SS}, based on average steady state plasma level; Cl_B, total body clearance.

Table 13: Comparative pharmacokinetics of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg. kg^{-1}) in healthy and febrile buffalo calves.

Parameter	Unit	Mean ±SE	
		Healthy	Febrile
α_1	h^{-1}	3.64 ± 0.15	5.67 ± 1.32
A_1	$\mu g.ml^{-1}$	9.17 ± 0.26	6.81 ± 0.38**
$t_{½}\ \alpha_1$	h	0.19 ± 0.008	0.17 ± 0.04
α_2	h^{-1}	0.38 ± 0.02	0.94 ± 0.07**
A_2	$\mu g.ml^{-1}$	2.97 ± 0.23	3.52 ± 0.37
$t_{½}\alpha_2$	h	1.84 ± 0.11	0.75 ± 0.05**
B	$\mu g.ml^{-1}$	0.93 ± 0.08	0.75 ± 0.17
β	h^{-1}	0.06 ± 0.003	0.08 ± 0.01*
$t_{½}\beta$	h	12.6 ± 0.77	9.32 ± 0.63**
AUC	$\mu g.h.ml^{-1}$	27.4 ± 0.48	14.0 ± 1.93**
AUMC	$\mu g.\ h^2\ .ml^{-1}$	197.8 ± 6.23	71.2 ± 11.9**
Vd_{area}	$L.kg^{-1}$	1.33 ± 0.08	2.15 ± 0.36
Vd_B	$L.kg^{-1}$	2.24 ± 0.21	3.46 ± 0.74
Vdss	$L.kg^{-1}$	0.53 ± 0.009	0.76 ± 0.07*
Cl_B	$L.kg^{-1}.h^{-1}$	0.07 ± 0.001	0.16 ± 0.02**

The values given are mean ± SE of results obtained from 6 animals.

* Mean significant difference (P<0.05) and ** mean significant difference (P<0.01).
α_1, distribution rate constant first; A_1, zero-time plasma drug concentration intercept of the least square regression line of distribution phase first; $t_{½}\alpha_1$, distribution half-life first; α_2, distribution rate constant second; A_2, zero-time plasma drug concentration intercept of the least square regression line of disribution phase second; $t_{½}\alpha_2$, distribution half life second ; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}\beta$, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; Vd_B, volume of distribution based on dose; Vd_{SS}, based on average steady state plasma level; Cl_B, total body clearance.

concentration at steady-state (Toutain and Bousquet-Melou 2004). Volume of distribution at steady state (Vd_{ss} = 0.76 ± 0.07 $L.kg^{-1}$) was significantly greater in buffalo calves induced with fever than healthy one (Vd_{ss} = 0.53 ± 0.009 $L.kg^{-1}$). The values indicated enhanced distribution of the parent drug and its metabolite in various body fluids and tissues in febrile condition. Similarly the value of Vd_{area} (2.15 ± 0.36 $L.kg^{-1}$) was comparatively greater in animals induced with fever than healthy one (Vd_{area} = 1.33 ± 0.08 $L.kg^{-1}$) but the difference between two groups was not statistically significant. However significant higher Vd_{ss} of ceftiofur in feverish cows than healthy was reported (Tohamy 2008).

Significantly increased body clearance (Cl_B = 0.16 ± 0.02 $L.kg^{-1}.h^{-1}$) of ceftiofur along with DFC in support to reduced distribution half life, added evidence to faster drug elimination in febrile animals compared to healthy one (Cl_B = 0.07 ± 0.001 $L.kg^{-1}.h^{-1}$). In corroboration with larger body clearance, significantly shorter elimination half-life ($t_{½}\beta$=9.32 h) was observed after single i.v. injection of ceftiofur sodium in feverish buffalo calves than healthy calves ($t_{½}\beta$ =12.6 h). Similar trend of significant increase in Cl_B and relatively longer $t_{1/2}\beta$ was observed in feverish cows as compared to healthy after i.v. administration of ceftiofur sodium (Tohamy 2008). When administered intravenously, there might be decreased ability of enzyme system to hydrolyse ceftiofur to DFC resulting in rapid renal elimination of parent drug in febrile buffalo calves. Endotoxin produces hepatic and renal dysfunction as well as hemodynamic depression. As hepatic function is significantly altered, the activities of various enzymes, responsible for the metabolism of antimicrobials, are altered, changing the elimination moreover biotransformation pattern of drugs during fever (Joshi and Sharma 2009). The altered metabolism during fever might be one of the reasons for rapid elimination of ceftiofur in feverish buffalo calves in the present study.

iii) *In vitro* plasma protein binding

It has been reported that several disease conditions may alter the protein binding and disrupt the usual relationship between free drug concentration and further pharmacological response. Disease state may affect protein binding through two main mechanisms, (i) by altering the concentration of proteins available for drug binding and (ii) by altering the affinity of drugs for plasma proteins (Sharma 2000).

Table 14 represents the extent of *in vitro* binding of ceftiofur with plasma proteins of buffalo calves, in which fever was induced with *E. coli* endotoxin. The extent of plasma protein binding varied from 34.9 - 46.2 % in feverish buffalo calves with an overall mean of 39.8 ± 1.28 %. Significant change in the plasma protein binding of ceftiofur was not observed in febrile buffalo calves as compared to healthy animals (40.9 %). Binding pattern of ceftiofur to plasma proteins was not concentration dependent in febrile buffalo calves. The binding capacity of drug to plasma proteins (βi) and dissociation rate constant of protein drug complex (K_β) of the drug-protein complex quantitatively describe the drug protein interaction. The βi and K_β were 2.40 ± 0.12 x 10^{-5} mole/g and 2.10 ± 0.09 x 10^{-6} mole, respectively. The higher value of βi than K_β indicated that binding of ceftiofur to plasma proteins was faster than dissociation of protein drug complex in buffalo calves. There was nonsignificant decrease in the βi and corresponding non significant increase in the K_β as compared to healthy animals indicates neglisible changes in the drug protein interaction in febrile animals. Findings of the protein binding of ceftiofur in febrile buffalo calves was similar to that reported by Chaudhary *et al* (1999) and Sharma (2000).

Table 14: Kinetic constants of *in vitro* ceftiofur binding to plasma proteins of febrile buffalo calves (n=6)

Expt. No.	Protein binding (%) Ceftiofur concentration (µg/ml)					β_i (10^{-5} mole/g)*	K_β (10^{-6} mole)**
	1	3	4	5	10		
1	38.6	37.5	35.7	46.2	40.8	2.20	1.90
2	40	40.9	32.6	44.1	34.9	2.37	2.10
3	37.4	41.8	41.6	42.8	42	2.61	2.22
Mean ± SEM	38.7 ± 0.75	40.1 ± 1.31	36.6 ± 2.64	44.4 ± 1.00	39.2 ± 2.19	2.40 ± 0.12	2.10 ± 0.09

* Association rate constant; ** Dissociation rate constant
Overall (mean ± SEM) binding = 39.8 ± 1.28 %

(C) Pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in hepatic dysfunctioned buffalo calves

i) Induction of hepatic dysfunction

Liver is the primary organ for majority of anabolic, catabolic as well as storage processes. It plays an important role in the biotransformation of xenobiotics by which toxic metabolites transformed into less harmful products and get excreted so as to reduce toxicity. However, during overdose these intermediate metabolites are produced in a large quantity resulting in liver cell damage and produce toxicity to this organ. Liver damage may lead to loss of efficacy and/or an augmented risk of toxicity in certain circumstance. During hepatic dysfunction, physiological changes occurring in the liver may alter the absorption, distribution, metabolism and elimination of antimicrobial drugs. It has been reported that hepatic dysfunction alters the pharmacokinetics of cephalosporins *viz*. ceftazidime (Sharma and Ul Haq 2012b), cefotaxime (Sharma *et al* 2005), cefuroxime (Kumar 1999) and fluoroquinolones like ciprofloxacin and pefloxacin in buffalo calves (Saini 1998). So, it was decided to investigate the effect of liver dysfunction on the pharmacokinetics and *in vitro* plasma protein binding of ceftiofur in hepatic dysfunctioned buffalo calves.

Effect of repeated administration of paracetamol/acetaminophen on the plasma activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Amylase are presented in table 15. Alterations in the plasma levels of Gamma glutamyl transferase (GGT), Cholesterol and Albumin are depicted in table 16. Significant increase in the plasma ALT activity was recorded with maximum of 446 % on day 3 after repeated administration of paracetamol. Similarly there was significant increase in the activity of AST in buffalo calves reaching peak on 4^{th} day (398.4 %) following paracetamol dosing.

Hepato-toxicity is the most noteworthy feature of acetaminophen overdose and it resembles other types of acute inflammatory liver diseases with significant increase in levels of ALT and AST (Payasi *et al* 2010). High plasma concentrations of ALT and AST are considered as an index of liver damage (Grewal *et al* 2009). Elevated levels of ALT in the plasma often indicate the existence of medical problems for instance hepatitis, liver damage, bile duct problems, congestive heart failure, infectious mononucleosis or myopathy and diabetes. AST is a cytoplasmic and mitochondrial enzyme released from cytoplasm of hepatocytes during degenerative changes (Evans and Health 1998). It is commonly measured clinically as a marker for liver health. The increase in AST activity after paracetamol overdose may be because of its release from ruptured hepatocytes or escape through membranes following altered permeability (Sharma and Ul Haque 2012b). The serum ALT is one of the most sensitive biochemical markers for the diagnosis of hepatic dysfunction (Johnkennedy *et al* 2010). During liver damage the transport function of hepatocytes is disturbed, resulting in consequential leakage of hepatic enzymes through plasma membrane. Therefore, an elevation of aminotransferase activity in the extracellular fluid or plasma is considered as a sensitive indicator for hepatocellular damage after paracetamol administration (Gill *et al* 2014a). The significant increase in the plasma

Table 15: Effect of repeated administration of acetaminophen on plasma activity of Alanine aminotransferase, Aspatate aminotransferase, Alkaline phosphatase and Amylase in buffalo calves.

Time after acetaminophen administration (days)	Alanine amino-transferase (IU/L)	Aspartate amino-transferase (IU/L)	Alkaline phosphtase (IU/L)	Amylase (IU/L)
0	16.3±0.95 (100)	106.0±3.64 (100)	89.3±2.54 (100)	13.8±1.30 (100)
1	68.7±1.94** (421.5)	321.8±3.40** (303.6)	101.0±3.01** (113.1)	9.00±0.68** (65.2)
2	62.8±1.68** (385.3)	381.5±5.84** (359.9)	107.3±2.11** (120.2)	7.67±0.49** (55.6)
3	72.7±1.93** (446.0)	401.2±3.82** (378.5)	115.5±1.77** (129.3)	7.33±0.42** (53.1)
4	63.6±2.36** (390.2)	422.3±4.70** (398.4)	122.3±1.84** (137.0)	7.50±0.43** (54.3)
5	61.7±1.48** (378.5)	379.7±4.70** (358.2)	118.8±1.78** (133.0)	6.83±0.48** (49.5)
6	59.0±2.67** (362.0)	325.0±5.14** (306.6)	114.7±1.71** (128.4)	6.67±0.33** (48.3)

The values given are mean ± SE of results obtained from 6 animals.

Values in parentheses indicate the percentage of corresponding increase/decrease (0 day values taken as 100 per cent).

* Mean significant difference (P<0.05) and ** mean significant difference (P<0.01 compared to control (0 day value).

Table 16: Effect of repeated administration of acetaminophen on plasma levels of Gamma glutamyl transferase, Cholesterol and Albumin in buffalo calves.

Time after acetaminophen administration (days)	Gamma glutamyl transferase (U/L)	Cholesteol (mg/dl)	Albumin (g/L)
0	13.8±0.48(100)	68.8±6.90(100)	3.08±0.12 (100)
1	16.8±0.60*(121.7)	59.2±6.11**(86.0)	2.72±0.07**(88.3)
2	18.8±0.70**(136.2)	49.3±6.43**(71.7)	2.43±0.04**(78.9)
3	21.0±0.73**(152.2)	47.6±6.60**(69.2)	2.30±0.02**(74.7)
4	19.8±0.60**(143.5)	48.6±5.65**(70.6)	2.33±0.03**(75.6)
5	16.5±0.56*(119.6)	47.0±6.97**(68.3)	2.39±0.03**(77.6)
6	15.3±0.71(110.9)	40.9±6.81**(59.4)	2.44±0.03**(79.2)

The values given are mean ± SE of results obtained from 6 animals.
Values in parentheses indicate the percentage of corresponding increase/decrease (0 day values taken as 100 per cent).
* Mean significant difference (P<0.05) and ** mean significant difference (P<0.01 compared to control (0 day value).

ALT and AST activities of paracetamol treated buffalo calves from day 1 to day 6 in the present study indicates liver impairment following its repeated administration.

Alkaline phosphate (ALP) is a zinc-containing enzyme present in liver, biliary tracts, small intestines, bones, lungs and kidney. The activity of enzyme was increased significantly from day 1 onward with maximum elevation on day 4 (137.0%). Elevated levels in blood may indicate damage to the liver and other organs (Sharma *et al* 2005 and Gill *et al* 2014b). The enhanced activity may be due to increased synthesis in response to hepatic damage as seen by the ALT levels in these buffalo calves after repeated administration of acetaminophen (Sharma and Ul-Haq 2012b).

The activity of amylase was significantly decreased from day 1 onwards with maximum decline of 48.3% on day 6 after repeated intramuscular administration of paracetamol. Serum amylase is an important marker for acute pancreatitis and the

reduced activity of this enzyme was reported after diazinon induced acute pancreatitis in rats (Gokalpa *et al* 2005). Similarly in the present study, the levels of amylase were reduced significantly in buffalo calves. The studies on pancreatectomized rats have already demonstrated the distribution of amylase in liver (Mcgeachin *et al* 1958). In addition abnormal serum activity in patients with hepatic disease postulated that liver can be one of the sources of serum amylase (Bhutta and Rahman 1971). Hepatic dysfunction as observed through increased ALT, AST and ALP levels might be secondary reason for decreased amylase activity in the present study. Thus significant decrease in the amylase activity in the present investigation might be because of reduced synthesis from damaged pancreatic and/hepatic cells after repeated administration of acetaminophen at high doses.

Repeated administration of paracetamol produced significant elevation in GGT from day 1 onwards with maximum (152.2 %) on 3rd day of treatment. GGT plays an important role in the gamma-glutamyl cycle - a pathway for the synthesis and degradation of glutathione during detoxification of drug and xenobiotics. Elevated serum GGT activity can be noted during liver, biliary and pancreatic diseases (Braun *et al* 1987). Serum GGT activity is used as a diagnostic criterion for hepatobiliary disease in cattle, sheep and horses (Gill 2014b and Radostits *et al* 2000). Increased GGT activity in the present study might be due to cholestatic malfunctioning following repeated paracetamol administration in buffalo calves (Gill *et al* 2014a). Results of the present investigation was in agreement with previous reports that subchronic administration of toxic doses of paracetamol produced significant increase in the activity of GGT in rats and mice respectively (Iyanda and Adeniyi 2011 and Darbar *et al* 2011).

Lipids are the key cellular entities which are not the only constituents of cell membrane but also play an important role in many cellular functions, metabolic

processes as well as in energy production. Liver is the main organ involved in the synthesis of lipoproteins, metabolism of cholesterol and hence alterations in plasma lipids level could be sensitive marker for assessing liver disorders (Muthulingam *et al* 2010). Cholesterol is esterified in liver and is used for the synthesis of bile acids and steroid hormones in the body. The process of esterification is affected during severe liver dysfunctions and produces hypocholesterolemia (Brar *et al* 2000). Repeated administration of paracetamol in overdose resulted in significant decline in the blood cholesterol levels from day 1 in buffalo calves with maximum of 59.4 % on 6^{th} day. Liver plays an active role in the metabolism of cholesterol so disruption of liver function in present study might be responsible for the decreased cholesterol levels in the present study. The decline in the blood cholesterol was in accordance with significant increase in the activity of plasma ALT, AST, ALP and significant decrease in the activity of amylase and GGT in the present investigation following repeated administration of paracetamol. Hypercholesterolaemia after repeated administration of paracetamol in buffalo calves was proposed due to entry of volatile fatty acids in the circulation following liver damage in addition to decreased metabolism/excretion of cholesterol (Sharma *et al* 2005). The contradictory results to that of of present study were observed in rats after paracetamol overdose (Madkour and Abdel-Daim 2013). They proposed that alterations of biomembrane lipid profile disturbs its fluidity and increases microviscosity resulting into hypercholestorelemia. Similar observation was recorded in rabbits (Al-Jowari 2011) stating that O_2^- or its dismutation product, H_2O_2 generated during the cytochrome P450-mediated microsomal metabolism of acetaminophen might be responsible for the increase in lipid peroxidation (LPO). The increase in the LPO activity in liver induced by acetaminophen produces tissue damage and thus release of cholesterol in the blood.

When acetaminophen was administered repeatedly, there was significant decrease in the plasma albumin levels with maximum of 74.7 % on 3rd day of administration (table 16). Liver is the major site for synthesis of albumin. The decrease in the albumin might be due to the liver damage as observed through significant increase in the activity of plasma ALT, AST, ALP and significant decrease in the activity of amylase and GGT after repeated administration of paracetamol in buffalo calves. Our results of decreased levels of albumin in the plasma of paracetamol (APAP) treated buffalo calves are in agreement with the observation of Iyanda and Adeniyi (2011). Similar statistical decline in serum albumin was reported upon chronic administration of paracetamol at high dose (Lebda *et al* 2013 and Madkour and Abdel-Daim 2013).

On the basis of daily analysis of different biochemical parameters on day 6 it was confirmed that liver dysfunction was fully induced in buffalo calves and pharmacokinetic study was planned on 7th day.

ii) Plasma levels and pharmacokinetics of ceftiofur and DFC in hepatic dysfunctioned buffalo calves

To determine the pharmacokinetics of ceftiofur in liver dysfunctioned buffalo calves, ceftiofur sodium was administered intravenously at the dose rate of 2 $mg.kg^{-1}$ body weight after induction of liver dysfunction. The plasma concentrations of ceftiofur at different time points after single intravenous administration in hepatic dysfunctioned buffalo calves are given in table 17. The logarithm of mean plasma ceftiofur and DFC concentration versus time curves in hepatic dysfunctioned buffalo calves were best fitted into a three compartment open model similar to healthy as well as febrile animals (Fig 12). Parent drug and DFC were detected up to 36 h post treatment in the plasma of hepatic dysfunctioned animals. Comparative plots of mean plasma concentration-time profile of ceftiofur and its metabolite following single

Table 17: Plasma levels (μg.ml^{-1}) of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in hepatic dysfunctioned buffalo calves.

Time (h)	Animals						Mean±SE
	1	2	3	4	5	6	
0.04	9.49	10.8	10.3	10.0	10.1	9.88	10.1 ± 0.21
0.08	6.05	7.49	8.06	7.39	8.11	7.49	7.43 ± 0.37
0.17	5.11	6.80	7.67	7.14	7.07	6.66	6.74 ± 0.44
0.25	4.39	6.24	6.55	7.16	6.61	6.25	6.20 ± 0.47
0.50	3.38	6.11	5.95	4.39	5.85	5.01	5.11 ± 0.54
0.75	2.56	4.77	4.77	2.73	3.34	3.61	3.63 ± 0.48
1.00	1.77	3.72	2.85	1.84	2.35	2.44	2.50 ± 0.36
2.00	0.96	1.92	2.32	1.14	1.92	1.61	1.64 ± 0.26
3.00	0.63	1.20	1.25	0.78	0.91	0.93	0.95 ± 0.12
4.00	0.54	0.99	0.91	0.70	0.82	0.80	0.79 ± 0.08
5.00	0.40	0.84	0.64	0.56	0.46	0.55	0.57 ± 0.08
6.00	0.35	0.67	0.53	0.41	0.36	0.41	0.45 ± 0.06
8.00	0.30	0.48	0.45	0.36	0.29	0.33	0.37 ± 0.04
10.0	0.28	0.41	0.42	0.32	0.24	0.30	0.33 ± 0.04
12.0	0.22	0.34	0.36	0.26	0.22	0.26	0.28 ± 0.03
16.0	0.17	0.28	0.25	0.21	0.15	0.20	0.21 ± 0.02
24.0	0.13	0.18	0.17	0.15	0.13	0.14	0.15 ± 0.01
36.0	0.10	0.11	0.10	0.09	0.11	0.12	0.11 ± 0.00

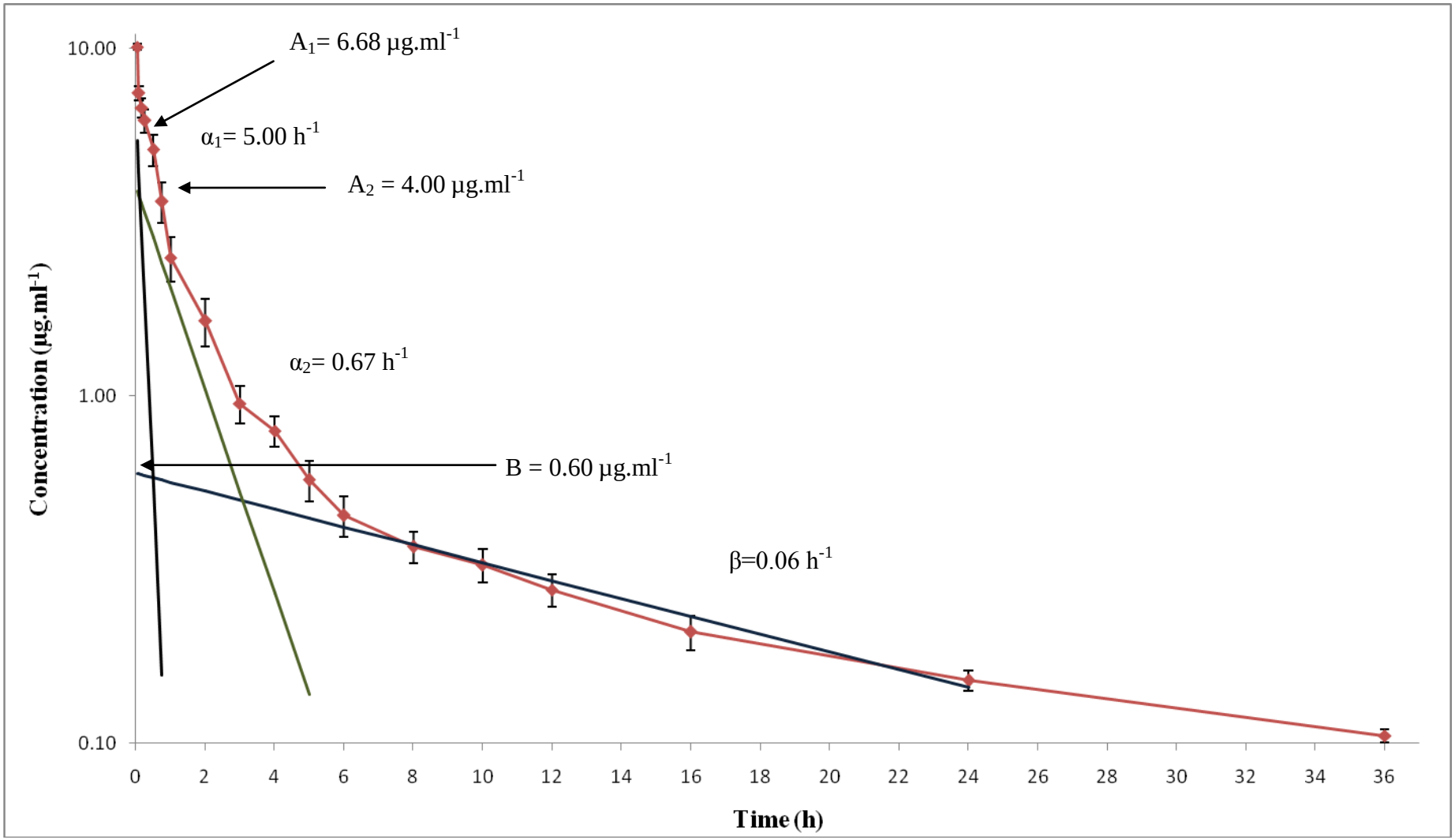


Fig. 12: Semilogarithmic plot of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in hepatic dysfunctioned buffalo calves.

intravenous administration of ceftiofur sodium (2 $mg.kg^{-1}$ B.W.) in healthy and hepatic dysfunctioned buffalo calves are depicted in figure 13. On comparing plasma levels of ceftiofur with healthy animals, it was observed that values were lower in buffalo calves with hepatic-dysfunction. The data can be adequately described by triexponential equation given below

$$C_P = A_1e^{-\alpha 1t} + A_2e^{-\alpha 2t} + Be^{-\beta t}$$

Where, C_P is ceftiofur and DFC concentration in plasma at time 't'. A_1, A_2, are zero time intercepts of distribution phases of log concentration time curve and B is zerotime intercepts of elimination phase of log concentration time curve, respectively. The α_1, α_2 are the distribution rate constants and and β is elimination rate constant, respectively.

The pharmacokinetic parameters for ceftiofur and its metabolite DFC following intravenous administrations of ceftiofur sodium in hepatic dysfunctioned buffalo calves are presented in table 18. Comparative pharmacokinetics of ceftiofur and desfuroylceftiofur in healthy and hepatic dysfunctioned buffalo calves are given in table 19. The significantly higher values of the distribution rate constant α_2 (0.67 ± 0.04 h^{-1}) in hepatic dysfunctioned buffalo calves indicated that ceftiofur was rapidly distributed into various body fluids and tissue compartments than healthy animals (α_2 = 0.38 ± 0.02 h^{-1}). Although initial distribution was more in diseased animals as observed by greater α_1, but it was nonsignificant compared to healthy animals. The disribution of ceftiofur in present study was in agreement with that observed after ceftazidime and cefotaxime administration respectively in hepatic dysfunctioned buffalo calves (Sharma and Ul Haq 2012b and Sharma *et al* 2005). Alterations in the permeability of biological membrane barrier and/or tissue during hepatic dysfunction

Table 18: Pharmacokinetic parameters of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in hepatic dysfunctioned buffalo calves.

Parameter (Unit)	Animals						Mean ±SE
	1	2	3	4	5	6	
α_1 (h^{-1})	6.80	6.04	4.81	2.90	4.72	4.69	5.00 ± 0.55
A_1 (µg.ml^{-1})	7.77	6.99	3.92	8.19	6.38	6.82	6.68 ± 0.61
$t_{½}$ α_1 (h)	0.10	0.11	0.14	0.24	0.15	0.15	0.15 ± 0.02
α_2 (h^{-1})	0.76	0.63	0.82	0.54	0.66	0.63	0.67 ± 0.04
A_2 (µg.ml^{-1})	2.73	4.46	6.55	1.99	4.52	3.75	4.00 ± 0.65
$t_{½}\alpha_2$ (h)	0.91	1.11	0.85	1.29	1.06	1.09	1.05 ± 0.07
B (µg.ml^{-1})	0.46	0.84	0.77	0.55	0.43	0.54	0.60 ± 0.07
β (h^{-1})	0.05	0.07	0.06	0.06	0.05	0.06	0.06 ± 0.00
$t_{½}\beta$ (h)	12.8	10.5	10.8	12.0	12.7	11.8	11.8 ± 0.40
AUC (µg.h.ml^{-1})	15.7	24.2	22.7	17.5	19.8	19.7	19.9 ± 1.29
AUMC (µg. h^2.ml^{-1})	112.9	162.8	148.8	119.1	124.1	131.1	133.1 ± 7.78
Vd_{area} (L.kg^{-1})	2.35	1.25	1.37	1.98	1.85	1.73	1.76 ± 0.17
Vd_B (L.kg^{-1})	4.38	2.39	2.61	3.61	4.60	3.68	3.54 ± 0.37
Vdss (L.kg^{-1})	0.91	0.56	0.58	0.78	0.63	0.68	0.69 ± 0.06
Cl_B (L.kg^{-1}.h^{-1})	0.13	0.08	0.09	0.11	0.10	0.10	0.10 ± 0.007

The values given are mean ± SE of results obtained from 6 animals.

α_1, distribution rate constant first; A_1, zero-time plasma drug concentration intercept of the least square regression line of distribution phase first; $t_{½}$ α_1, distribution half-life first; α_2, distribution rate constant second; A_2, zero-time plasma drug concentration intercept of the least square regression line of disribution phase second; $t_{½}\alpha_2$, distribution half life second ; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}\beta$, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; Vd_B, volume of distribution based on dose; Vd_{SS}, based on average steady state plasma level; Cl_B, total body clearance.

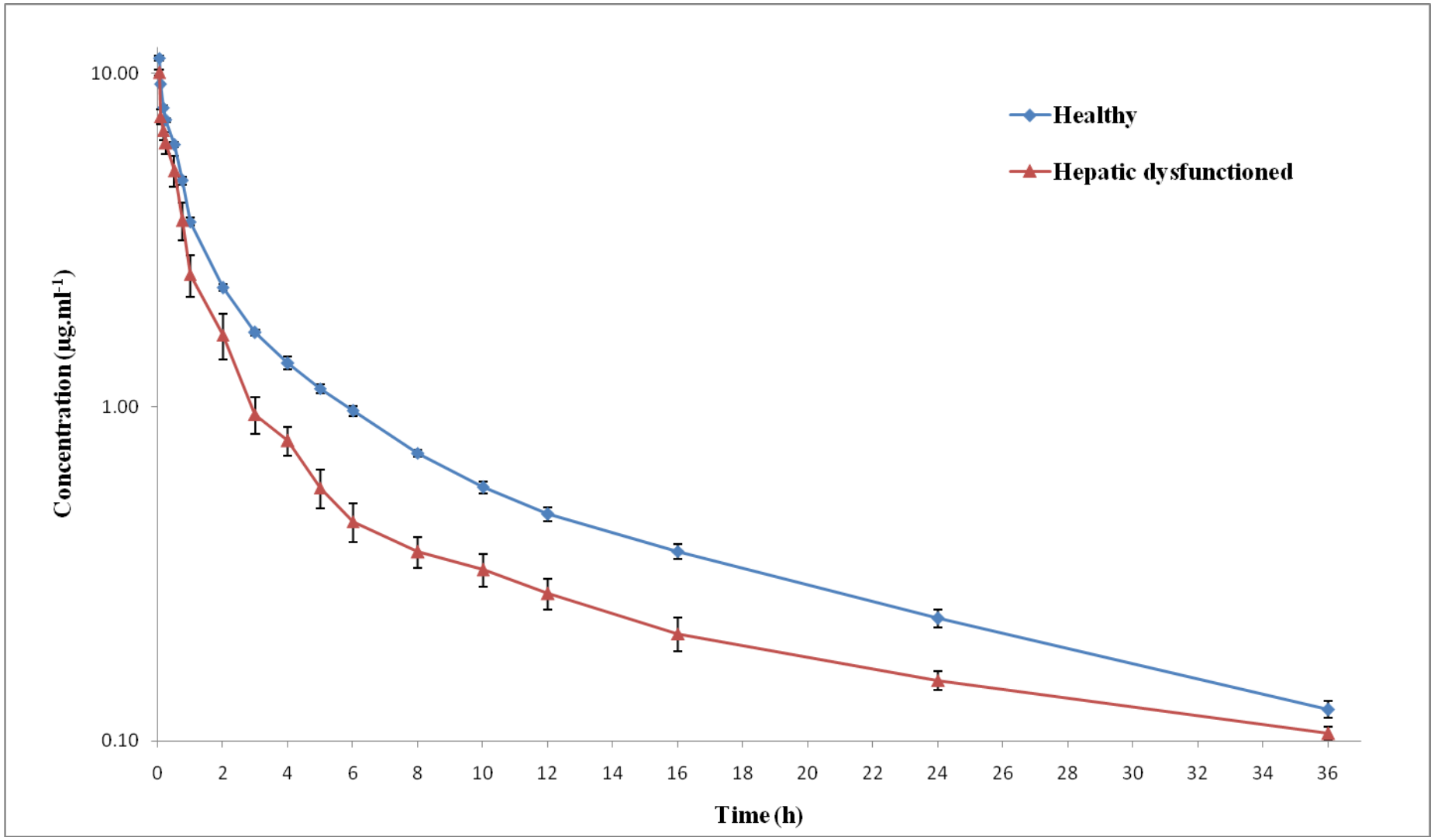


Fig. 13: Comparative plots of mean plasma concentration-time profile of ceftiofur and desfuroylceftiofur following single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy and hepatic dysfunctioned buffalo calves.

Table 19: Comparative pharmacokinetic of ceftiofur and desfuroylceftiofur after single intravenous administration of ceftiofur sodium (2 mg.kg^{-1}) in healthy and hepatic dysfunctioned buffalo calves.

Parameter	Unit	Mean ±SE	
		Healthy	Hepatic dysfunctioned
α_1	h^{-1}	3.64 ± 0.15	5.00 ± 0.55
A_1	$\mu g.ml^{-1}$	9.17 ± 0.26	6.68 ± 0.61**
$t_{½}\ \alpha_1$	h	0.19 ± 0.008	0.15 ± 0.02
α_2	h^{-1}	0.38 ± 0.02	0.67 ± 0.04**
A_2	$\mu g.ml^{-1}$	2.97 ± 0.23	4.00 ± 0.65
$t_{½}\alpha_2$	h	1.84 ± 0.11	1.05 ± 0.07**
B	$\mu g.ml^{-1}$	0.93 ± 0.08	0.60 ± 0.07*
β	h^{-1}	0.06 ± 0.003	0.06 ± 0.00
$t_{½}\beta$	h	12.6 ± 0.77	11.8 ± 0.40
AUC	$\mu g.h.ml^{-1}$	27.4 ± 0.48	19.9 ± 1.29**
AUMC	$\mu g.\ h^2\ .ml^{-1}$	197.8 ± 6.23	133.1 ± 7.78**
V_{darea}	$L.kg^{-1}$	1.33 ± 0.08	1.76 ± 0.17
V_{dB}	$L.kg^{-1}$	2.24 ± 0.21	3.54 ± 0.37*
Vdss	$L.kg^{-1}$	0.53 ± 0.009	0.69 ± 0.06*
Cl_B	$L.kg^{-1}.h^{-1}$	0.07 ± 0.001	0.10 ± 0.007**

The values given are mean ± SE of results obtained from 6 animals.

* Mean significant difference (P<0.05) and ** mean significant difference (P<0.01).

α_1, distribution rate constant first; A_1, zero-time plasma drug concentration intercept of the least square regression line of distribution phase first; $t_{½}\ \alpha_1$, distribution half-life first; α_2, distribution rate constant second; A_2, zero-time plasma drug concentration intercept of the least square regression line of disribution phase second; $t_{½}\alpha_2$, distribution half life second ; B, zero-time plasma drug concentration intercept of the least square regression line of elimination phase; β, elimination rate constant; $t_{½}\beta$, elimination half-life; AUC, area under concentration–time curve; AUMC, area under the first moment curve; Vd_{area}, apparent volume of distribution based on AUC; $Vd_{B,}$ volume of distribution based on dose; Vd_{SS}, based on average steady state plasma level; Cl_B, total body clearance.

might be responsible for change in distribution pattern of ceftiofur in hepatic dysfunctioned buffalo calves (Madkour and Abdel-Daim 2013 and Al-Jowari 2011).

The value of area under curve (AUC) represents the plasma exposure of drug and thus its antimicrobial activity against susceptible pathogens. Significantly decreased AUC (19.9 ± 1.29 μg.h.ml^{-1}) in hepatic dysfunctioned buffalo calves reported in the present study indicate that less area of the body was covered by ceftiofur and DFC concentration when compared to healthy calves (AUC=27.4 ± 0.48 μg.h.ml^{-1}).

The volume of distribution signifies the drug diffusion into the body tissues (Gilman *et a1* 2011 and Galinsky and Svensson 1995). The volume of distribution at steady state (Vd_{ss}) is the constant that expresses the amount of the drug in the body at steady state as a proportion of the corresponding expected plasma concentration at steady-state (Toutain and Bousquet-Melou 2004). Vd_{ss} (0.69 ± 0.06 L.kg^{-1}) and Vd_B (3.54 ± 0.37 L.kg^{-1}) were significantly greater in buffalo calves with hepatic dysfunction than healthy animals (Vd_{ss} = 0.53 ± 0.009 L.kg^{-1}, Vd_B = 2.24 ± 0.21 L.kg^{-1}) indicating better distribution of the parent drug and its metabolite in various body fluids and tissues in hepatic damage. Similar high volume of distribution was reported after i.v. administration of cefotaxime in hepatic dysfunctioned buffalo calves (Sharma *et al* 2005). Although values of Vd_{area} were more in diseased calves compared to healthy, it was not statistically significant.

Significantly increased body clearance (Cl_B= 0.10 ± 0.007 L.kg^{-1}.h^{-1}) of ceftiofur along with DFC in support to reduced distribution half life, added evidence to faster drug elimination in hepatic dysfunctioned buffalo calves compared to healthy one (Cl_B= 0.07 ± 0.001 L.kg^{-1}.h^{-1}). Similarly greater Cl_B of ceftazidime and cefotaxime was recorded when administered intravenously in buffalo calves (Sharma

and Ul Haq 2012b and Sharma *et al* 2005). Although body clearance was larger, elimination half-life ($t_{½}\beta$ = 11.8 ± 0.40 h) observed after single i.v. injection of ceftiofur sodium in hepatic dysfunctioned buffalo calves was similar to that of healthy calves ($t_{½}\beta$ = 12.6 ± 0.77 h). Similar finding was also reported in buffalo calves following i.v. administration of ceftazidime (Sharma and Ul Haq 2012b). It has been postulated that during hepatic dysfunction, the phase I mixed function oxidases are selectively affected i.e. cytochome P450s along with increased activities of phase II enzymes i.e glucuronyl transferase, acetyl transferase and sulfotransferase. The increased activities of these metabolizing enzymes might be one of the reasons for increased hepatic clearance of ceftiofur during hepatic dysfunction as a consequence of disease specific changes in both enzyme activity and/or drug transport within the liver (Sharma and Ul haq 2012b and Sharma *et al* 2005). It has also been stated that the activities of various enzymes responsible for the metabolism of antimicrobials are significantly altered during hepatic dysfunction (Joshi and Sharma 2009). The altered metabolism of ceftiofur during liver damage might be responsible for its rapid elimination in hepatic dysfunctioned buffalo calves in the present study.

iii) *In vitro* plasma protein binding

Table 20 depicts the extent of *in vitro* binding of ceftiofur with plasma proteins of buffalo calves, in which hepatic dysfunction was induced after repeated administration of paracetamol. The extent of plasma protein binding varied from 32.6 - 49.1 % in hepatic dysfunctioned buffalo calves with an overall mean of 42.1 ± 1.60 %. Significant change in the plasma protein binding of ceftiofur was not observed in buffalo calves after hepatic dysfunction as compared to healthy animals (40.9 %). Results of protein binding of ceftiofur are in accordance with that of cefotaxime in hepatic dysfunctioned buffalo calves (Sharma 2000).

Table 20: Kinetic constants of *in vitro* ceftiofur binding to plasma proteins of hepatic dysfunctioned buffalo calves (n=6)

Expt. No.	Protein binding (%) Ceftiofur concentration (µg/ml)					β_i (10^{-5} mole/g)*	K_β (10^{-6} mole)**
	1	3	4	5	10		
1	46.7	49.1	33.5	38.8	41.8	2.35	1.68
2	43.3	40.6	32.6	47.1	36.9	2.75	1.83
3	42.8	47.1	43.3	43.9	44.0	2.77	1.97
Mean ± SEM	44.3 ± 1.23	45.6 ± 2.57	36.5 ± 3.43	43.3 ± 2.42	40.9 ± 2.10	2.62 ± 0.14	1.83 ± 0.08

* Association rate constant; ** Dissociation rate constant
Overall (mean ± SEM) binding = 42.1 ± 1.60 %

Binding pattern of ceftiofur to plasma proteins was not concentration dependent in hepatic dysfunctioned buffalo calves. The binding capacity of drug to plasma proteins (βi) and dissociation rate constant of protein drug complex (K_β) of the drug-protein complex quantitatively describe the drug protein interaction. The βi and K_β were 2.62 ± 0.14 x 10^{-5} mole/g and 1.83 ± 0.08 x 10^{-6} mole, respectively. The higher value of βi than K_β indicated that binding capacity of ceftiofur to plasma proteins was faster than dissociation of protein drug complex in these buffalo calves. The nonsignificant increase in the binding capacity of ceftiofur with corresponding nonsignificant decrease in dissociation rate constant as compared to healthy animals (βi=2.54 x 10^{-5} mole/g, K_β=2.0 x 10^{-6} mole) might be due to decrease in plasma albumin levels after repeated administration of paracetamol but overall there was neglisible changes in the drug protein interaction in hepatic dysfunctioned buffalo calves. Comparative data of *in vitro* plasma protein binding of ceftiofur in healthy, febrile and hepatic dysfunctioned buffalo calves is presented in table 21.

Table 21: Comparative data of *in vitro* plasma protein binding of ceftiofur in healthy, febrile and hepatic dysfunctioned buffalo calves.

Parameter	Unit	Healthy	Febrile	Hepatic dysfunctioned
Extent of binding	%	40.9 ± 1.49^a	39.8 ± 1.28^a	42.1 ± 1.60^a
β_i	(10^{-5} mole/g)	2.54 ± 0.12^a	2.4 ± 0.12^a	2.62 ± 0.14^a
K_β	(10^{-6}mole)	2.00 ± 0.08^a	2.1 ± 0.09^a	1.83 ± 0.08^a

(D) Pharmacodynamics of ceftiofur

In vitro susceptibility testing of antimicrobials plays an important role in predicting its efficacy against bacterial pathogens. Often, the choice of an antimicrobial agent and its dosing is based on results of these susceptibility tests (Salmon *et al* 1996). Reports suggest that *E. coli* and *S. aureus* are most prevalent environmental contaminant in veterinary hospitals. Diarrhoea in young pre-weaned calves is one of the most important causes of calf morbidity and mortality. Hospital-acquired infections (HAI) are an important cause of morbidity and mortality in human as well as veterinary patients (Murphy *et al* 2010). The incidence of diarrhoea (bacterial scours) in calves under 30 days of age varies from 10-20%. Calf diarrhoea has an adverse effect on health status of newborn calves, longevity in the herd, productive performance and thus great economic losses in livestock sector and it is also one of the most frequent causes of septicaemia (Luna 2008).

S. aureus is highly prevalent infectious bacteria isolated from bovine mammary gland secretions. The success rate of combination of systemic treatments along with intramammary varies from 0 to 80% in treating infections caused by *Staphylococcal spp*. (Goji *et al* 2004). In dairy cattle, *S. aureus* is recurrently associated with subclinical mastitis and may cause decrease milk yield, contaminate

milk and alter quality of milk products thereby having a significant impact on livestock economy (Cervinkova *et al* 2013). It is an important foodborne pathogen with zoonotic potential causing a wide variety of diseases in humans and animals like skin infections, pneumonia and septicemia (Fagundes *et al* 2010 and Cervinkova *et al* 2013).

Taking into consideration the importance of these microbes, antimicrobial activity of ceftiofur was tested against *E. coli* and *S. aureus*. Antimicrobial activity of ceftiofur was assessed *in vitro* by determination of MIC and MBC of the drug after overnight incubation in MHB (Mueller Hinton Broth). The MIC is defined as the minimum concentration of antimicrobial that prevents the clear suspension of 10^6 CFU/ml from becoming turbid after overnight incubation. Turbidity usually connotes at least a 100-fold decrease in bacterial colony count at the end of incubation period when compared to growth control. The MIC of ceftiofur was 0.20 $\mu g.ml^{-1}$ and 0.35 $\mu g.ml^{-1}$ against reference strains of *E. coli* and *S. aureus*, respectively (table 22). The minimum concentration of antimicrobial that reduces the initial bacterial inoculum size by 1000 fold is considered as MBC. The values of MBC for ceftiofur against reference strains of *E. coli* and *S. aureus* were 0.30 $\mu g.ml^{-1}$ and 0.50 $\mu g.ml^{-1}$, respectively.

Table 22: MIC, MBC of Ceftiofur against *E. coli* and *S. aureus*.

Strain	MIC ($\mu g.ml^{-1}$)	MBC ($\mu g.ml^{-1}$)
E. coli (reference strain MTCC 739)	0.20	0.30
S. aureus (reference strain MTCC 737)	0.35	0.50

The range of MIC for ceftiofur reported against various pathogens of veterinary importance was in the range of 0.10 to 0.50 $\mu g.ml^{-1}$ which is in concurrence with the present investigation. The MIC of ceftiofur against reference

strain of *Pasteurella multocida* (B2:p52) and clinical isolates in MHB as well as serum was reported to be in the range of 0.1 – 0.23 μg.ml^{-1} (Singh 2014). The minimum inhibitory concentration (MIC_{90}) of ceftiofur reported for *Pasturella haemolytica*, *Pasturella multocida*, *Haemophilus somnus*, *Actinobacillus pleuropneumonia* and *Klebsiella pneumoniae* was 0.25 μg.ml^{-1} (Salmon *et al* 1996 and Deshpande *et al* 2000). Reported values of MIC_{90} of ceftiofur against common pathogenic microorganisms such as *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella melaninogenica* were in the range 0.125–0.5 μg.ml^{-1} (Witte *et al* 2011). In addition, MIC value of 0.2 μg.ml^{-1} was reported against the common respiratory pathogens like *Pasteurella multocida* and *Mannheimia haemolytica* by different groups of workers in cattle and goat, respectively (Berge *et al* 2006 and Dore *et al* 2011), whereas the range was 0.25-0.5 μg ml^{-1} for *Staphylococcus aureus* and *Streptococcus pneumoniae*. (Zhang *et al* 2008).

Time above the MIC is an important PK-PD parameter directly related to the clinical or *in vivo* efficacy of cephalosporins. For maximum efficacy to be reached, most crucial point is that the average unbound plasma drug concentration should be above the pathogen's MIC_{90} for at least 50-70% of the dosing interval (Drusano 1998). Ceftiofur has been reported to show time-dependent bacterial killing (Plumb 2008) therefore the length of duration for which concentration of ceftiofur and its metabolite remains above the MIC_{90} value is an important consideration (Craig 1995, Turnidge 1998 and McKellar *et al* 2004). For cephalosporin antibiotics, a T > MIC of 35-40% of the interdose interval has been recommended as optimal for bacteriostatic action while a T> MIC of 60-70% is obligatory for a bactericidal effect (Craig 1998 and Toutain *et al* 2002).

The calculated values of % T ≥ MIC for estimated and/or reported range of MICs *i.e.* 0.1 - 0.5 μg.ml^{-1} using interdose intervals 8, 12, 24, 36 and 48 h after single i.v., i.m. and s.c. administration of ceftiofur sodium (2 mg.kg^{-1} B.W.) in healthy buffalo calves are presented in table 23, 24 and 25, respectively. Since, % T > MIC values were 83.5%, 80.3% and 73.3% at dosing intervals 24, 24 and 36 h, respectively after single i.v., i.m. and s.c. administration of ceftiofur sodium at an MIC of 0.5μg.ml^{-1}, the selected dose can be repeated after these intervals, respectively. Thus, it indicates that the selected dose of ceftiofur sodium can be repeated at 24 h interval for i.v. and i.m. dosing and at 36 h interval for s.c dosing for effective treatment of the diseases caused by the microorganisms having MIC ≤ 0.5 μg.ml^{-1} in healthy buffalo calves.

Table 26 depicts the calculated values of % T ≥ MIC for estimated and/or reported range of MICs i.e. 0.1 - 0.5 μg.ml^{-1} using dosing intervals 8, 12, 24, 36 and 48 h after single i.v. administration of ceftiofur sodium (2 mg.kg^{-1} B.W.) in febrile buffalo calves. % T > MIC values were 83.5 % and 69.8 % at dosing intervals 24 and 12 h respectively after single i.v administration of ceftiofur sodium (2 mg.kg^{-1} B.W.) in healthy and febrile buffalo calves respectively. It indicates that the selected dose of ceftiofur sodium can be repeated at 24 h interval in healthy and 12 h interval in feverish buffalo calves following i.v. dosing of ceftiofur sodium at selected dose for effective treatment of the febrile conditions caused by the pathogens having MIC ≤ 0.5 μg.ml^{-1}. Hence, single i.v. injection of ceftiofur sodium in febrile buffalo calve is recommended to be repeated at reduced interval (12 h) for effective treatment of febrile conditions caused by these microorganisms compared to healthy calves (24 h).

Table 23: Time above minimum inhibitory concentration (%T > MIC) expressed as percentage of the inter-dose interval (8, 12, 24, 36, 48 h) after intravenous administration of ceftiofur (2 mg.kg^{-1} B.W.) in healthy buffalo calves.

Interdose interval (h)	MIC (µg.ml^{-1})							
	0.10	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**	**0.75**	**1**
8	615.5	458.3	366.3	331.3	301.0	250.4	158.5	93.2
12	410.3	305.5	244.2	220.9	200.7	167.0	105.6	62.1
24	205.2	152.8	122.1	110.4	100.4	**83.5**	52.8	31.1
36	136.8	101.8	81.4	73.6	66.9	55.7	35.2	20.7
48	102.6	76.4	61.0	55.2	50.2	41.7	26.4	15.5

Table 24: Time above minimum inhibitory concentration (%T > MIC) expressed as percentage of the inter-dose interval (8, 12, 24, 36, 48 h) after intramuscular administration of ceftiofur (2 mg.kg^{-1} B.W.) in healthy buffalo calves.

Interdose interval (h)	MIC (µg.ml^{-1})							
	0.10	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**	**0.75**	**1**
8	734.4	521.9	397.6	350.3	309.4	241.0	116.7	28.5
12	489.6	347.9	265.1	233.6	206.3	160.7	77.8	19.0
24	244.8	174.0	132.5	116.8	103.1	**80.3**	38.9	9.5
36	163.2	116.0	88.4	77.9	68.8	53.6	25.9	6.3
48	122.4	87.0	66.3	58.4	51.6	40.2	19.5	4.8

Table 25: Time above minimum inhibitory concentration (%T > MIC) expressed as percentage of the inter-dose interval (8, 12, 24, 36, 48 h) after subcutaneous administration of ceftiofur (2 mg.kg^{-1}B.W.) in healthy buffalo calves.

Interdose interval (h)	MIC (µg.ml^{-1})							
	0.10	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**	**0.75**	**1**
8	808.3	602.2	481.6	435.8	396.1	329.7	209.2	123.6
12	538.9	401.5	321.1	290.5	264.1	219.8	139.4	82.4
24	269.4	200.7	160.5	145.3	132.0	109.9	69.7	41.2
36	179.6	133.8	107.0	96.8	88.0	**73.3**	46.5	27.5
48	134.7	100.4	80.3	72.6	66.0	55.0	34.9	20.6

Table 26: Time above minimum inhibitory concentration (%T > MIC) expressed as percentage of the inter-dose interval (8, 12, 24, 36, 48 h) after intravenous administration of ceftiofur (2 mg.kg^{-1}B.W.) in febrile buffalo calves.

Interdose interval (h)	MIC (µg.ml^{-1})							
	0.10	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**	**0.75**	**1**
8	375.0	258.6	190.5	164.6	142.2	104.7	36.6	-11.8
12	250.0	172.4	127.0	109.7	94.8	**69.8**	24.4	-7.8
24	125.0	86.2	63.5	54.9	47.4	34.9	12.2	-3.9
36	83.3	57.5	42.3	36.6	31.6	23.3	8.1	-2.6
48	62.5	43.1	31.7	27.4	23.7	17.4	6.1	-2.0

The calculated values of % T ≥ MIC for estimated and/or reported range of MICs i.e. 0.1 - 0.5 μg.ml^{-1} using dosing intervals 8, 12, 24, 36 and 48 h after single i.v. administration of ceftiofur sodium (2 mg.kg^{-1}) in hepatic dysfunctioned buffalo calves are presented in table 27. % T > MIC values were 83.5 % and 58.2 % at 24 h dosing interval after single i.v administration of ceftiofur sodium (2 mg.kg^{-1}B.W.). It indicates that the selected dose of ceftiofur sodium can be repeated at 24 h interval in healthy as well as in hepatic dysfunctioned buffalo calves following i.v. dosing of ceftiofur sodium at selected dose for effective treatment of the diseases caused by the pathogens having MIC ≤ 0.5 μg.ml^{-1}. Hence, dosage regimen of ceftiofur sodium is same as that of healthy buffalo calves when given intravenously i.e. single dose of 2 mg.kg^{-1} B.W. repeated after 24 h.

Table 27: Time above minimum inhibitory concentration (%T > MIC) expressed as percentage of the inter-dose interval (8, 12, 24, 36, 48 h) a intravenous administration of ceftiofur (2 mg.kg^{-1} B.W.) in hepatic dysfunctioned buffalo calves.

Interdose interval (h)	MIC (μg.ml^{-1})							
	0.10	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**	**0.75**	**1**
8	516.0	369.0	282.9	250.2	221.9	174.6	88.5	27.5
12	344.0	246.0	188.6	166.8	147.9	116.4	59.0	18.3
24	172.0	123.0	94.3	83.4	74.0	**58.2**	29.5	9.2
36	114.7	82.0	62.9	55.6	49.3	38.8	19.7	6.1
48	86.0	61.5	47.2	41.7	37.0	29.1	14.8	4.6

CHAPTER V

SUMMARY

In the present investigation, the detailed study on pharmacokinetics of ceftiofur sodium was conducted in healthy, febrile and hepatic dysfunctioned buffalo calves along with *in vitro* plasma protein binding. The results of the PK studies indicate that ceftiofur followed three compartment open model after single i.v. administration and two compartment open model after single i.m. and s.c. administration in healthy buffalo calves. The absorption was fair in i.m. and s.c route. Longer distribution half life of drug irrespective of route indicated the persistence of the drug in the body of buffalo calves. Greater AUCs i.e. 27.4 ± 0.48 μg.h.ml^{-1}, 26.8 ± 0.75 μg.h.ml^{-1} and 36.2 ± 0.81 μg.h.ml^{-1} following i.v., i.m. and s.c. administration, respectively indicate better plasma exposure of ceftiofur sodium. The volume of distribution indicated better distribution of parent drug and its metabolite DFC in various body fluids and tissues irrespective of the route of administration. After i.v. administration, there might be decreased ability of enzyme system to hydrolyse ceftiofur to DFC resulting in rapid renal elimination of parent drug as compared to i.m. and s.c. administration. This might be one of the reasons that elimination half life is shorter after i.v. dosing ($t_{½}\beta$ = 12.6 ± 0.77 h) of ceftiofur sodium than i.m. ($t_{½}\beta$ = 17.0 ± 1.74 h) and s.c. ($t_{½}\beta$ = 16.5 ± 0.79 h) in buffalo calves.

During febrile state, the drug followed three compartment open model similar to that of healthy buffalo calves following i.v. dosing. It was observed that at most of the times plasma concentrations of ceftiofur and its metabolite were less in febrile animals as compared to healthy animals. The distribution of drug was rapid as indicated by significantly higher distribution rate constant in feverish calves (α_2= 0.94 ± 0.07 h^{-1}) than healthy ones (α_2= 0.38 ± 0.02 h^{-1}). Persistence of the drug was

reduced in febrile state ($t_{1/2}\alpha 2$ = 0.75 ± 0.05 h) than healthy ($t_{1/2}\alpha_2$ = 1.84 ± 0.11 h). Significantly lower AUC in febrile animals (14.0 ± 1.93 µg.h.ml^{-1}) pointed to reduced plasma exposure of ceftiofur and DFC as compared to healthy animals (27.4 ± 0.48 µg.h.ml^{-1}). In support to reduced distribution half life, significantly increased body clearance (Cl_B = 0.16 ± 0.02 L.kg^{-1}.h^{-1}) and shortened elimination half life ($t_{1/2}\beta$= 9.32 ± 0.63 h) in febrile buffalo calves indicated faster elimination of the drug as compared to healthy calves (Cl_B = 0.07 ± 0.001 L.kg^{-1}.h^{-1}, $t_{1/2}\beta$= 12.6 ± 0.77 h). This indicates that pharmacokinetics of ceftiofur in febrile state differs from that of healthy control.

The drug followed three compartment open model in hepatic dysfunctioned buffalo calves similar to that observed after i.v. administration in healthy animals. On comparing plasma levels of ceftiofur and its metabolite, it was found that concentrations were less at most of the times in diseased animals than healthy one. The drug was distributed rapidly into various body fluids and tissue compartment of hepatic dysfunctioned buffalo calves (α_2 = 0.67 ± 0.04 h^{-1}) as compared to healthy animals (α_2 = 0.38 ± 0.02 h^{-1}). Significantly shortened distribution half life ($t_{1/2}\alpha_2$= 1.05 ± 0.07 h) in diseased buffalo calves than healthy calves ($t_{1/2}\alpha_2$ = 1.84 ± 0.11 h) denoted reduced persistence of ceftiofur and DFC in the body. Plasma exposure (AUC = 19.9 ± 1.29 µg.h.ml^{-1}) of the drug was significantly reduced in animals with hepatic dysfunction as compared to healthy (AUC = 27.4 ± 0.48 µg.h.ml^{-1}). Greater distribution (Vd_{ss}= 0.69 ± 0.06 L.kg^{-1}, Vd_B = 3.54 ± 0.37 L.kg^{-1}) of drug and its metabolite was seen in hepatic dysfunctioned buffalo calves in comparision to healthy calves (Vd_{ss}= 0.53 ± 0.009 L.kg^{-1},Vd_B = 2.24 ± 0.21 L.kg^{-1}). Increased body clearance (Cl_B = 0.10 ± 0.007 L.kg^{-1}.h^{-1}) of parent drug along with its metabolite added the evidence of faster elimination from the body of animals with hepatic dysfunction than healthy animals (Cl_B= 0.07 ± 0.001 L.kg^{-1}.h^{-1}) in support to reduced distribution. The

results indicated that there are marked alterations in the pharmacokinetics of ceftiofur in buffalo calves following hepatic dysfunction.

Ceftiofur was found to be moderately protein bound (40.9 ± 1.49 %) in the plasma of healthy buffalo calves. The extent of protein binding was almost similar in febrile (39.8 ± 1.28 %) and hepatic (42.1 ± 1.60 %) dysfunctioned buffalo calves. Thus, there was no significant change in the plasma protein binding of ceftiofur in healthy as well as disease states.

Antimicrobial activity of ceftiofur was tested against the microorganisms of veterinary importance *viz. E. coli* and *S. aureus*. The MIC and MBC of the drug estimated against reference strain of *E. coli* was 0.20 $\mu g.ml^{-1}$ and 0.30 $\mu g.ml^{-1}$, respectively. The MIC and MBC of the antimicrobial against *S. aureus* were 0.35 $\mu g.ml^{-1}$ and 0.50 $\mu g.ml^{-1}$, respectively. The MICs of ceftiofur against the pathogens of veterinary importance tested and/or reported in the literature are in the range of 0.10 to 0.50 $\mu g.ml^{-1}$.

The satisfactory % T ≥ MIC values were maintained up to 24 after single i.v. and i.m. administration and 36 h after single s.c. administration of ceftiofur sodium (2 $mg.kg^{-1}$ B.W.) in healthy buffalo calves, respectively. Thus, selected dose of ceftiofur sodium can be repeated at 24 h interval for i.v. and i.m. dosing and at 36 h interval for s.c dosing for effective treatment of the diseases caused by the microorganisms having MIC ≤ 0.5 $\mu g.ml^{-1}$ in healthy animals.

In febrile animals % T > MIC value was 69.8 % at 12 h dosing intervals after single i.v administration of ceftiofur sodium (2 $mg.kg^{-1}$ B.W.) in febrile buffalo calves. This confirms that single i.v. injection of ceftiofur sodium in feverish buffalo calve is recommended to be repeated at reduced interval (12 h) for effective treatment

of febrile conditions caused by microorganisms having MICs ≤ 0.5 μg.ml^{-1} compared to healthy calves (24 h).

The calculated values of % T ≥ MIC for estimated and/or reported range of MICs i.e. ≤ 0.5 μg.ml^{-1} was 58.2 % at dosing intervals of 24 h after single i.v. administration of ceftiofur sodium (2 mg.kg^{-1} B.W.) in hepatic dysfunctioned buffalo calves. Hence dosage regimen of ceftiofur sodium is same as that of healthy buffalo calves in hepatic dysfunctioned animals when given intravenously i.e. single dose of 2 mg.kg^{-1} B.W. repeated after 24 h.

Conclusion

On the basis of the information gathered in the present investigation, it is concluded that both fever and hepatic dysfunction significantly altered the pharmacokinetics and dosage regimen of ceftiofur sodium in buffalo calves. The resulting dosage regimen should be followed while treating buffalo calves with fever or hepatic dysfunction caused by bacterial pathogens with an MIC ≤ 0.5 μg.ml^{-1}.

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