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Bioequivalence Study of Two Different Formulations of Ceftiofur Following Intramuscular Administration in Cattle

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Abstract

The aim of the present study was to explore the bioequivalence of ceftiofur hydrochloride sterile suspension (5%) in two formulations, a reference formulation (Excenel® Ready To Use (RTU) 5% Ceftiofur (CEF) (Pfizer, New Jersey, USA)) and a test formulation (ceftipure 5% (Alke, Istanbul, Turkey)). Both products were administered to each of 10 healthy Holstein cattle (1.1 mg/kg body weight, intramuscularly) during a two-period crossover parallel experimental design. Blood samples were collected before and at 0.16, 0.33, 0.5, 1, 2, 4, 8, 12, and 24 hours after a single intramuscular administration. The plasma concentrations of ceftiofur and desfuroylceftiofur-related metabolites were measured by high-performance liquid chromatography. The descriptive pharmacokinetic parameters were calculated and compared by variance analysis, with 90% confidence intervals. The comparison values between reference and test formulation for maximum plasma concentration, time to maximum concentration, area under the plasma concentration-time curve to last concentration, and area under the plasma concentration-time curve extrapolated to infinity were 0.59 ± 0.15 µg/mL, 0.53 ± 0.20 µg/mL, 2.10 ± 0.30 hours, 2.00 ± 0.00 hours, 2.94 ± 0.13 µg h/mL, 2.84 ± 0.25 µg h/mL, and 3.16 ± 0.19 µg h/mL, 3.10 ± 0.14 µg h/mL, respectively. In addition, 90% CIs of these ratios for reference and test product were within acceptable ranges, and the relative bioavailability (F) of test products was 96.57% according to area under the plasma concentration-time curve to last concentration. The results demonstrated that ceftipure 5% is bioequivalent to Excenel®RTU 5% CEF in cattle.

Keywords: Bioequivalence, cattle, ceftiofur

Introduction

Substandard and counterfeit medicines are notable concerns, especially in countries which lack a rigorous regulatory authority, and they may undermine the confidence of physicians and consumers in generic drugs. However, the high pricing of drugs provided through official channels pushes patients to purchase drugs in non-regulated industries (such as street markets) where counterfeit and substandard drugs are common. Therefore, making affordable and quality-assured generic medicines accessible through public and private-regulated channels are an important issue. Proving that a generic product is therapeutically equivalent through bioequivalence (BE) studies can be accepted as an indicator of its quality assurance and can be regarded as interchangeable with the original brand product in terms of efficacy and safety (Cameron et al., 2012; Cetin & Arıcıoğlu, 2009). BE studies are scientific methods designed for the purposes of monitoring the pharmacokinetic and pharmacodynamic parameters of drugs tested in the pharmaceutical industry and comparing different series and administration routes (Palermo-Neto & Righi, 2008; Rita & Akhilesh, 2015). The main goal of BE studies that are crucial for the development of a pharmaceutical preparation in the pharmaceutical industry is to evaluate the therapeutic compatibility of the drugs tested (pharmaceutical equivalents or pharmaceutical alternatives) (Vetchý et al., 2007). In parallel with the large increase in the production and consumption of generic products, which are generally 20%–80% cheaper than the original and thus allow savings in health expenditures, BE studies have gained more importance in the last decade (Cetin & Arıcıoğlu, 2009).

In veterinary medicine, BE studies have special importance as they allow the establishment of the necessary conditions for the registration of generic animal health products that provide animal and food

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safety for humans (Palermo-Neto & Righi, 2008) With this respect, in many countries around the world, legal regulations have been introduced in BE studies for medicines used in veterinary as well as for human since 1990 (Schall & Endrenyi, 2010). The guidelines for the determination of drug BE have been written by the regulatory authorities of the European Community or the United States of America (Toutain & Koritz, 1997). In this context, in the "Bioequivalence Study Guide for Veterinary Medicinal Products" published by the European Medicines Agency (EMA) in 2011, the design of BE studies for veterinary drugs and the evaluation criteria to be taken into account when making BE decisions are specified. According to the guidelines of EMA, the comparison of pharmacokinetic parameters between two formulations is the best method for a BE examination of veterinary drugs, in which the maximum plasma concentration (C_{max}), the area under the plasma concentration-time curve to last concentration (AUC_{0-t}), the area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), and time to maximum concentration (Tmax) are used for bioequivalent analysis (European Medicines Agency [EMA], 2011; Xiong et al., 2018). The BE between two formulations is demonstrated when the clinical efficacy of the test formulation is equivalent to those detected in clinical trials of the reference formulation (Lei et al., 2017).

Ceftiofur is a third-generation broad-spectrum cephalosporin, which is widely used to treat respiratory diseases in ruminants, horses, swine, and poultry (Jacobson et al., 2006; Xiong et al., 2018). It exhibits strong antibacterial activity against both gram-negative and gram-positive bacteria, including β-lactamase-producing strains (Al-Kheraije, 2013).

Ceftiofur is approved in Europe and the United States to treat bovine respiratory diseases induced by *Mannheimia haemolytica, Pasteurella multocida*, and *Haemophilus somnus*. The dosage of ceftiofur hydrochloride salt prepared as a sterile suspension is 1 mg ceftiofur equivalent (CE)/kg once a day for 3 days in Europe and 1.1–2.2 mg CE/kg body weight for 3–5 days in the United States (Kausche & Robb, 2003). Ceftiofur is rapidly metabolized in most animal species after parenteral administration, and its active metabolite is desfuroylceftiofur. In desfuroylceftiofur, the integrity of the β-lactam ring, which is essential for antimicrobial activity, is preserved (Hornish & Kotarski, 2002).

Several studies have been conducted evaluating the pharmacokinetic properties of ceftiofur in cattle (Altan et al., 2017; Brown et al., 2000; Wang et al., 2018). The aim of establishing BE for generic products is to exhibit equivalence in pharmacokinetic parameters between reference and test products (EMA, 2011). Therefore, these studies are significant in the development of new pharmaceutical formulations (Mestorino et al., 2016).

This study was aimed to explore the BE of ceftiofur hydrochloride (5%) sterile suspension in two formulations, a reference formulation (Excenel®RTU 5% CEF (Pfizer, New Jersey, USA)) and a test formulation (ceftipure 5% (Alke, Istanbul, Turkey)).

Methods

Chemicals

Ceftiofur hydrochloride (>95% purity) and desfuroylceftiofur (>98% purity) were purchased from Sigma (St. Louis, MO, USA). All solvents for high-performance liquid chromatography (HPLC) and other reagents were used as analytical grade.

Drugs

For BE study, two different commercial products of ceftiofur injection containing 5% of ceftiofur hydrochloride were used, one for the test product (ceftipure 5% (Alke)) and one for the reference product (Excenel®RTU 5% CEF (Pfizer). Both formulations were previously analyzed to confirm ceftiofur concentrations.

Animals

In this study, all animal studies were approved by İstanbul University Animal Experiments Ethics Committee, İstanbul, Turkey (Date: September 30, 2010, Approval No:2010/152). Ten Holstein male cattle, 9–13 months old and weighing 175–340 kg were provided from a private cattle breeding farm of Bursa Province (Turkey).

Previously to the beginning of the study, cattle were clinically examined to confirm their health status and they were identified by using plastic numbered ear-tags. The animals were kept in clean and disinfected paddocks of the farm from which they were supplied during the study and were fed with cattle-rearing feed. All animals were allowed to acclimatize for 1 week prior to the study, and no drug/chemical administration was applied to animals before the injection of ceftiofur preparations. In this study, the animal numbers, administration route, dose, and study design were in accordance with the other studies (Brown et al., 2000; El-Gendy et al., 2007).

Sample Collection and Experimental Design

The study was carried out according to a two-sequential crossover design with a washout period of 14 days. The animals were randomly divided into two groups, group A and group B, with five cattle in each group. Cattle in group A received ceftipure 5%, (Alke) in the first period and Excenel®RTU 5% CEF, (Pfizer) in the second period. The drugs were given to the cattle in group B in reverse order. Reference and test products were administered via intramuscular route (1.1 mg/kg body weight). Blood samples of 5 mL were taken in lithium heparin tubes (Vacuette® Heparin Tubes ,Greiner Bio-One, Frickenhausen, Germany) from the jugular vein, before drug administration and at 0.16, 0.33, 0.5, 1, 2, 4, 8, 12, and 24 hours after administration. The blood samples were centrifuged at 1500 rpm for 15 minutes to separate the plasma samples and then stored at −20°C until analyzed by HPLC.

Ceftiofur and Desfuroylceftiofur-Related Metabolites Analysis

Plasma samples were transferred frozen to ARGEFAR (Research and Application Center of Drug Development and Pharmacokinetics)/Ege University for analysis. Plasma desfuroylceftiofur (DFC) concentrations were analyzed by using a Prominence UFLC (Ultra-Fast Liquid Chromatographic system (Shimadzu, Tokyo, Japan) HPLC with ultraviolet (UV) detector (Knauer, UVD 2.1S) according to the methods described by Jacobson et al. (2006) and De Baere et al. (2004), with slight modifications. Separation was achieved using a Zorbax Octadecylsilyl (ODS) column (250 × 4.6 mm, 5 μm i.d.; Agilent Technologies, Santa Clara, CA, USA). The mobile phase conditions consisted of mobile phase A (0.1% trifluoroacetic acid (v/v) in water) and mobile phase B (0.1% trifluoroacetic acid (v/v) in acetonitrile) (1:1, v/v). The column oven was set at 40°C. The flow rate and injection volumes were 1.5 mL/min and 50 μL, respectively. Detection and quantification were conducted at a wavelength of 254 nm.

After injection of ceftiofur, it is rapidly converted to the biologically active metabolite, DFC. Therefore, the pharmacokinetics of ceftiofur was determined by the plasma DFC concentrations. Ceftiofur is

extracted from plasma samples using a derivatization method that converts ceftiofur and all metabolites to DFC. In the first step, the plasma samples (500 µL) were deproteinized by adding extraction solution (7 mL) prepared as 0.4% (w/v) dithioerythritol (1,4-dithioerythritol) in borate buffer solution and vortexed for 3 minutes. The samples were kept in a 50°C water bath for 15 minutes. During the waiting period, the vortexing process was applied at regular intervals of 3 minutes, and the samples were put back into the water bath. In the second step, 1.5 mL of iodoacetamide solution was added to the samples that were left to cool down at room temperature (15 minutes) and vortexed and left for 30 minutes in a dark, no light environment. During the dwell period, the solid-phase extraction cartridges (Chromabond columns easy volume: 3 mL, content of sorbent: 60 mg material: Polypropylene (PP)) were activated. The samples were allowed to pass slowly through the cartridge under vacuum. The resulting residue was dried under mild nitrogen vapor. To the dried tubes, 500 µL of water were added and vortexed for 15 seconds. The solution obtained was transferred to vials, and 50 µL of the solution was injected into the HPLC-UV.

Method Validation

Calibration samples were prepared from a 1 mg/mL stock solution of desfuroylceftiofur by diluting 0.6 µg/mL and 20 µg/mL solutions of desfuroylceftiofur and were analyzed by HPLC. Inter and intraday accuracy and precision values were estimated by assaying control plasma containing three different concentrations of 0.6 µg/mL, 1 µg/mL, and 10 µg/mL of desfuroylceftiofur. Accuracy was expressed as % RSD (relative standard deviation) and precision as % CV (coefficient of variation).

The sensitivity of the method was examined by the measurement of the lower limit of detection (LOD) and limit of quantitation (LOQ). Detection limit was estimated from a signal-to-noise ratio of 3.

Pharmacokinetic, Bioequivalence, and Statistical Analysis

Descriptive pharmacokinetic parameters were obtained with WinNonlin Professional software (WinNonlin® Professional Version 4.1, Pharsight Corporation, Scientific Consulting Inc., North Carolina, USA).

C_{max} and T_{max} were obtained using the plasma concentration versus time data. Area Under Curve (AUC) was calculated by the linear trapezoidal rule until the last sampling time ($AUC_{0-24\ h}$) and with extrapolation to infinity ($AUC_{0-\infty}$).

The pharmacokinetic parameters excluding T_{max} were logarithmically transformed before the data analysis, based on EMA 2001 bioequivalence guidelines for veterinary drug. T_{max} comparison was performed with non-parametric tests based on NPar Mann–Whitney test. Pharmacokinetic parameters were compared between Excenel®RTU 5% and ceftipure 5% with analysis of variance by independent samples *t*-test using Statistical Package for the Social Sciences (SPSS) statistical software (SPSS 15.0, Chicago, IL, USA). The BE acceptance criteria were that the 90% CI of the difference between the reference formulation and the test formulation for the variables AUC_{0-t} and $AUC_{0-\infty}$ ranged within 80%–125%. The acceptance limits for C_{max} were wider than of AUC, with a range of 70%–143% (European Generic Medicines Association [EGMA], 2010; EMA, 2011; United States Food and Drug Administration [FDA], 2003). CIs were calculated with SPSS analysis. *p*-value of less than .05 was considered statistically significant (*$p < .05$ and **$p < .01$).

Results

All cattle remained in a healthy state throughout the experiments, and no adverse reactions were recorded. The amounts of active substance of Excenel®RTU 5% CEF (Pfizer) and Ceftipure 5%, (Alke) measured before the study, were obtained as 53.2 mg/mL and 50.65 mg/mL, respectively. It was determined that the difference between the active ingredient amounts of the reference and test product was less than 5% as stated in the BE study guide for veterinary medicinal products published by EMA in 2011 (EMA, 2011).

DFC Analysis in Plasma by HPLC

The calibration curves were in good linearity over the range of 0.6–20 µg/mL, with a correlation coefficient of 0. 9947. The LOD and LOQ values of method were determined as 0.19 µg/mL and 0.58 µg/mL, respectively.

The chromatogram of a cattle plasma sample showing the desfuroylceftiofur peak after intramuscular injection of ceftiofur (1.1 mg/kg, body weight) is illustrated in Figure 1A. The chromatogram acquired from an extract of the drug-free plasma sample is presented in Figure 1B. Under expressed chromatographic conditions, the retention time was approximately 6.1 minutes for desfuroylceftiofur.

The intraday and interday variation recovery, precision, and accuracy of the method are represented in Tables 1 and 2, respectively. The mean recovery was within the range of 99.7%–106.59%, precision and accuracy for the 0.6 µg/mL, 1 µg/mL, and 10 µg/mL were all below 7.8% in plasma.

Pharmacokinetic Analysis

A comparison of the mean plasma concentration-time curves (arithmetic and semilogarithmic) of ceftiofur and DFC after intramuscular administration of each formulation (Excenel®RTU 5% CEF (Pfizer) and ceftipure 5% (Alke)) are presented in Figure 2.

The mean pharmacokinetic parameters (log-transformed and untransformed) for two formulations (Excenel®RTU 5% CEF (Pfizer) and ceftipure 5% (Alke)) are presented in Table 1. Pharmacokinetic parameters did not reveal any statistically significant difference between the two products. The relative bioavailability of ceftipure 5% (Alke) compared to Excenel®RTU 5% CEF (Pfizer) was determined as 96.57% according to AUC_{0-t} (Table 3).

Bioequivalence Analysis

In order to determine the BE of the reference and test products, C_{max} and AUC were regarded as the main parameters. BE was assessed by determining 90% CIs of the ratio of test/reference formulation, using log-transformed data. The mean values of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ (after log-transformation of data) and CIs analysis results of ceftipure 5% (Alke) and Excenel®RTU 5% CEF (Pfizer,) are illustrated in Table 4. Based on the data of two one-sided *t*-test, there was no statistically significant difference in C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ parameters between test and reference formulations. The 90% CIs values of the test formulation in $AUC_{0-\infty}$ and AUC_{0-t} were 91.96%–102.43% and 80.85%–114.88%, respectively, which is within the BE range (80%–125%) of the reference product. The 90% CIs values in C_{max} ranged between 74.03% and 100.84%, which also was within the BE range (70%–143%) of the reference formulation (Table 4).

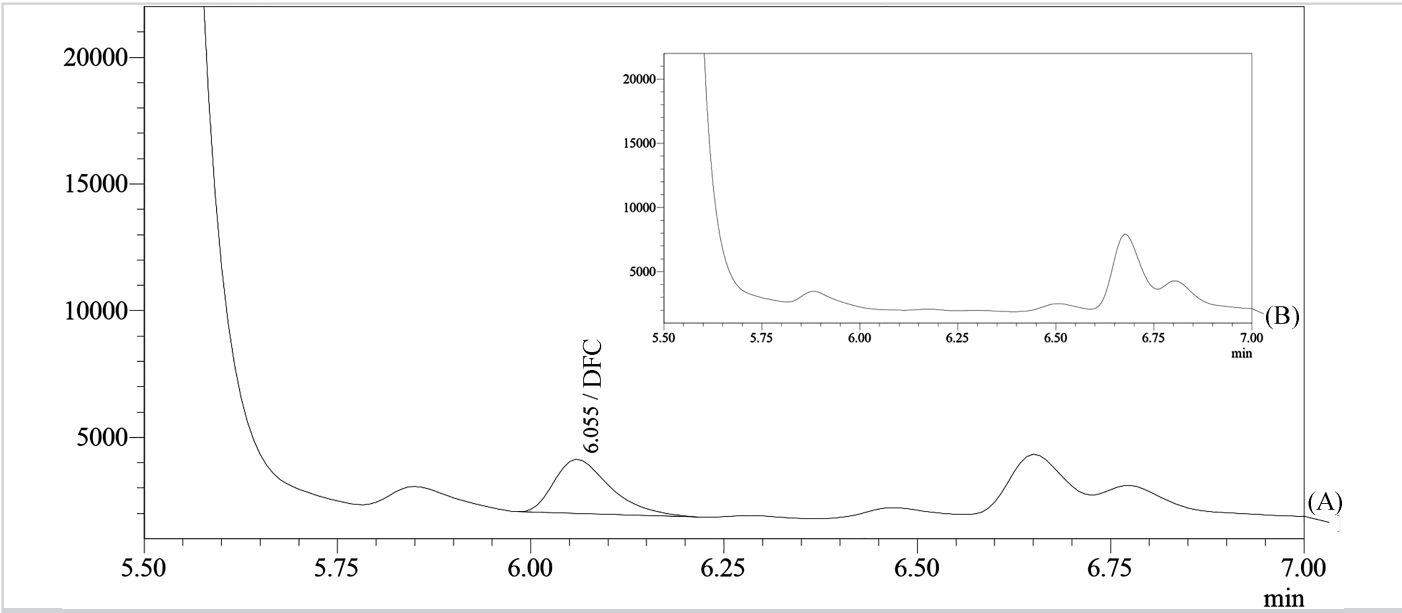

Figure 1
Chromatogram (A) of a Cattle Plasma Sample Illustrating the Desfuroylceftiofur Peak Following the Administration of Ceftiofur 1.1 mg/kg Bodyweight. Inset (B) Shows a Chromatogram of Blank Cattle Plasma.

Discussion, Conclusion and Recommendations

Antibiotics are antimicrobial compounds that are used in both human medicine and animal agriculture to reduce incidences of diseases (Peng et al., 2014). The supply of these drugs in reliable and quality standards is very important for human and animal health. Otherwise, the substandard medicines can cause poisoning, untreated disease, early death, treatment failure, and an increase in antimicrobial resistance problems (Ozawa et al., 2018).

The analytical method showed good specificity, linearity, accuracy, and precision for the quantitation of desfuroylceftiofur in plasma samples, thus allowing its use in BE assays. In this study, the pharmacokinetic properties of two veterinary medicinal products (Excenel® RTU 5% CEF

Table 1
Intraday Recovery, Precision, and Accuracy

Desired (µg/mL)	Calculated (µg/mL)	Recovery (%)	Mean	SD	Precision (%CV)	Accuracy (%RSD)
0.600	0.618	103.000				
0.600	0.623	103.833				
0.600	0.627	104.500				
0.600	0.623	103.833				
0.600	0.615	102.500	103.533	0.785	0.758	3.533
1.000	1.009	100.900				
1.000	1.004	100.400				
1.000	1.013	101.300				
1.000	0.999	99.900				
1.000	1.004	100.400	100.580	0.536	0.533	0.580
10.000	9.995	99.950				
10.000	10.026	100.260				
10.000	9.966	99.660				
10.000	10.038	100.380				
10.000	10.102	101.020	100.254	0.512	0.511	0.254

% RSD = (calculated concentration−desired concentration)/(desired concentration × 100); % CV = (SD/calculated concentration)× 100.
CV, coefficient of variation; RSD, relative standard deviation; SD, standard deviation.

Table 2

Interday Recovery, Precision, and Accuracy

Desired (µg/mL)	Day 1 (Calculate)	% Recovery	Day 2 (Calculate)	% Recovery	Day 3 (Calculate)	%Recovery	Mean (%Recovery)	SD (%Recovery)	%CV (%Recovery)	Accuracy (%RSD)
0.60	0.615	102.500	0.694	115.667	0.601	100.167	106.111	8.36	7.876	
0.60	0.624	104.000	0.694	115.667	0.611	101.833	107.167	7.42	6.927	
0.60	0.622	103.667	0.699	116.500	0.596	99.333	106.500	8.93	8.382	
Mean	0.620	103.389	0.696	115.944	0.603	100.444	106.593			
SD	0.005	0.788	0.003	0.481	0.008	1.273	0.436			
%RSD	0.762	0.762	0.415	0.415	1.267	1.267	7.723			7.723
%CV	3.389	3.389	15.944	15.944	0.444	0.444	0.409			
1.00	0.996	99.600	1.032	103.200	0.965	96.500	99.767	3.35	0.003	
1.00	0.999	99.900	1.030	103.000	0.964	96.400	99.767	3.30	0.003	
1.00	1.006	100.600	1.018	101.800	0.963	96.300	99.567	2.89	0.003	
Mean	1.000	100.033	1.027	102.667	0.964	96.400	99.700			
SD	0.005	0.513	0.008	0.757	0.001	0.100	0.094			
%RSD	0.513	0.513	0.738	0.738	0.104	0.104	3.156			3.156
%CV	0.033	0.033	2.667	2.667	−3.600	−3.600	0.095			
10.00	10.090	100.900	10.199	101.990	9.662	96.620	99.797	2.84	0.003	
10.00	10.114	101.140	10.196	101.960	9.660	96.600	99.833	2.89	0.003	
10.00	10.124	101.240	10.204	102.040	9.649	96.490	99.860	3.00	0.003	
Mean	10.109	101.093	10.200	101.997	9.657	96.570	99.830			
SD	0.017	0.175	0.004	0.040	0.007	0.070	0.026			
%RSD	0.173	0.173	0.040	0.040	0.072	0.072	2.911			2.911
%CV	1.093	1.093	1.997	1.997	−3.430	−3.430	0.026			

%RSD = (calculated concentration−desired concentration)/(desired concentration × 100); %CV = (SD/calculated concentration) × 100.
CV, coefficient of variation; RSD, relative standard deviation; SD, standard deviation.

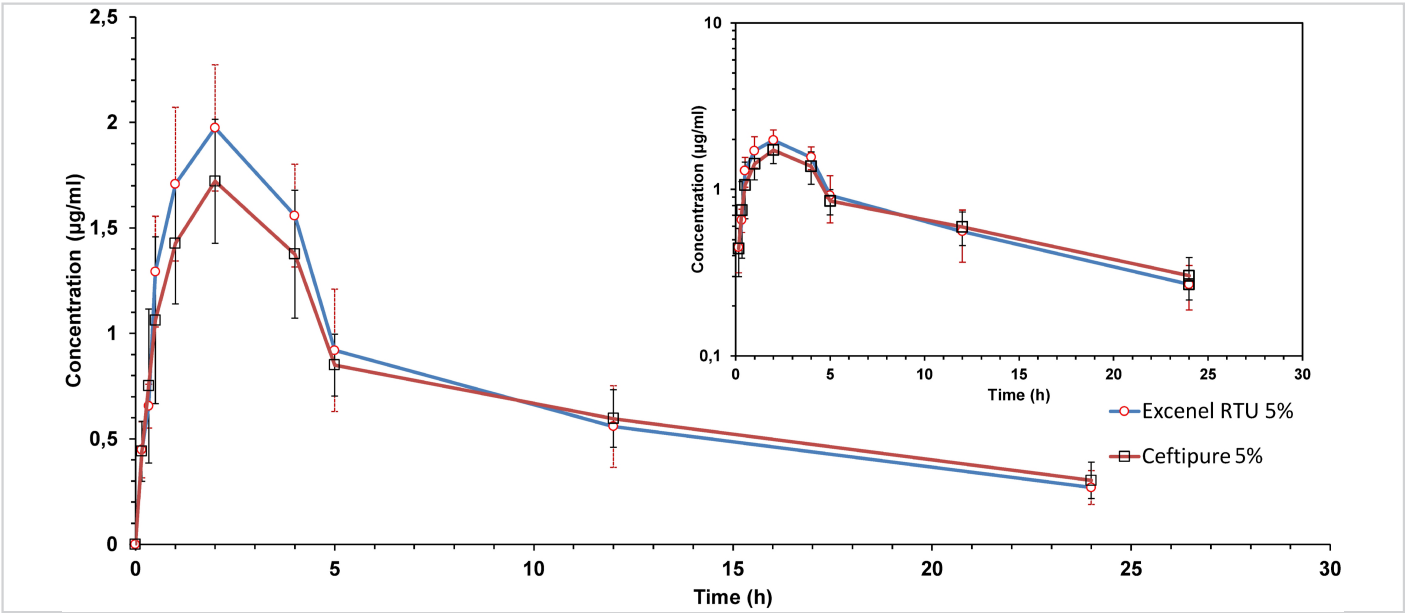

Figure 2
Comparative Plasma Concentration Versus Time Curves (Means ± SD) of Ceftiofur and DFC in Plasma After Single-Dose Intramuscular Administrations of Two Formulation (Excenel®RTU 5%, Pfizer and ceftipure 5%, Alke) to Healthy Aattle. Small Inserted Figure; Semilogarithmic Comparative Curves (0–24 hours After Each Administration). DFC, Desfuroylceftiofur; SD, standard deviation.

(Pfizer) and ceftipure 5% (Alke) containing the active ingredient ceftiofur at a level of 5% were compared, and the BE potential of these formulations was evaluated. The mean plasma profile acquired for both products as well as the pharmacokinetic parameters was determined to be similar.

AUC is a useful metric that expresses the total amount of drug that comes into the systemic circulation after drug administration (Gupta, 2018). In our study, the values of AUC_{0-t} were 17.92 ± 4.03 µg h/mL and 19.25 ± 2.69 µg h/mL for test and reference product, respectively. The AUC_{0-t} values were considerably smaller compared to those reported by El-Gendy at al. (2007) in Friesian and Buffalos calves receiving 2.2 mg/kg body weight dose (intravenous and intramuscular) of ceftiofur (67.712 ± 4.98 µg h/mL and 34.700 ± 1.85 µg h/mL, respectively). Considering the AUC_{0-t} values, the relative bioavailability of the ceftipure 5% to the Excenel®RTU 5% was 96.57%, which satisfied the requirements of EMA (2011). The C_{max} values of the test and the reference products were 1.81 ± 0.35 µg/mL and 1.86 ± 0.26 µg/mL, respectively, and this was quite lower than those previously reported for cattle (13.9 ± 3.55 µg/mL). The high C_{max} and AUC values determined in other studies are thought to be due to the administration of different dosage forms of cetiofur to

Table 3
Pharmacokinetic Parameters (Mean ± SD) of Ceftiofur and DFC After Single Intramuscular Administration of the Reference (Excenel®RTU 5%, Pfizer) and Test (Ceftipure 5%, Alke) Formulations to Cattle

Parameters	Unit	Test (UT)	Reference (UT)	Test (LT)	Reference (LT)
AUC_{0-t}	µg h/mL	17.92 ± 4.03	19.25 ± 2.69	2.84 ± 0.25	2.94 ± 0.13
$AUC_{0-\infty}$	µg h/mL	22.55 ± 3.11	24.13 ± 5.04	3.10 ± 0.14	3.16 ± 0.19
C_{max}	µg/mL	1.81 ± 0.35	1.86 ± 0.26	0.53 ± 0.20	0.59 ± 0.15
T_{max}	hour	2.00 ± 0.00	2.10 ± 0.30	2.00 ± 0.00	2.10 ± 0.30
$AUMC_{0-t}$	µg h^2/mL	149.48 ± 37.79	164.21 ± 34.21	4.96 ± 0.30	5.08 ± 0.19
$AUMC_{0-\infty}$	µg h^2/mL	386.02 ± 237.82	361.23 ± 150.95	5.83 ± 0.42	5.80 ± 0.38
MRT	hour	8.29 ± 0.78	8.48 ± 0.79	2.11 ± 0.10	2.13 ± 0.10
$T_{1/2}$	hour	13.38 ± 11.09	10.33 ± 2.01	2.38 ± 0.49	2.31 ± 0.09
F	%			96.57	

UT, untransformed data; LT, log-transformed data; SD, standard deviation.
AUC_{0-t}, area under the plasma concentration-time curve from zero to the last point; $AUC_{0-\infty}$, area under the concentration-time curve from zero to infinity; C_{max}, maximum plasma concentration; T_{max}, time to reach maximum plasma concentration; $AUMC_{0-t}$, area under the first moment of curve from zero to the last point; $AUMC_{0-\infty}$, area under the first moment of curve from zero to infinity; MRT, mean residence time; $T_{1/2}$, the half life of elimination; F, the relative bioavailability.

Table 4

Two One-Sided t*-Test and 90% CI Results of the Parameters After a Single* İ*ntamuscular Administration of the Reference (Excenel®RTU 5%, Pfizer) and Test (Ceftipure 5%, Alke) Formulations*

Parameters	Test (T)	Reference (R)	ANOVA	90% CI	Ratio (T/R) (%)	Acceptable Range (%)
AUC_{0-t}	2.84 ± 0.25	2.94 ± 0.13	0.265	80.85–114.88	96.57	80–125
$AUC_{0-\infty}$	3.10 ± 0.14	3.16 ± 0.19	0.237	91.96–102.43	98.10	80–125
C_{max}	0.53 ± 0.20	0.59 ± 0.15	0.718	74.03–100.84	89.83	70–143

cattle. Beta-lactam antibiotics exert time-dependent bactericidal activity, which is determined by the free-drug concentration-time above the minimum inhibitory concentration (MIC) for the causative organism (Masich et al., 2018). Plasma ceftiofur concentrations in 24 hours after the administration of test and reference formulations were 0.30 ± 0.08 µg/mL and 0.26 ± 0.08 µg/mL, respectively. The concentrations of 0.30 µg/mL and 0.26 µg/mL are more than at least four times the MIC (≤0.06 µg/mL) for ceftiofur against major microorganisms such as *M. haemolytica*, *H. somnus,* and *P. multocida* (Brown et al., 2000). Therefore, we concluded that both formulations could be beneficial for a therapeutic effect lasting 24 hours. In this study, we also determined the values of other pharmacokinetic parameters such as area under the first moment of curve and mean residence time for both Excenel®RTU 5% CEF (Pfizer) and ceftipure 5% (Alke), and we revealed that there was no statistical difference in the parameters between the products.

We evaluated the potential of ceftipure 5% (Alke) to be equivalent to Excenel®RTU 5% CEF (Pfizer) in line with the recommendations in the "Bioequivalence Study Guide for Veterinary Medicinal Products" published by EMA (2011). For BE decision, we selected C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ as primary pharmacokinetic parameters, and after log transformation of the data (except T_{max}), all parameters were compared statistically. We observed that there were no statistically significant changes between the reference and test formulations in C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$. The C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ results presenting 90% CI were also in the equivalence interval of 80%–125% (70–143 for C_{max}) set by the European Union. Similarly, there was no significant difference in T_{max} value between the two products.

The results of our study confirm that ceftipure 5% (Alke) is bioequivalent to Excenel®RTU 5% CEF (Pfizer) and can be safely used interchangeably.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University (Date: September 30, 2010, Approval No: 2010/152).

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