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The Investigation of Bioequivalence of Some Enrofloxacin Preparations Following Oral Administration in Broilers

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Abstract
This study evaluated the bioequivalence of three different enrofloxacin preparations in broilers after oral administration. Forty male broilers (Ross 308 broiler breeders) fed with feed containing no residues of drugs and pollutants for 30 days were used in the study. They were divided into four experimental groups each consisting of 10 animals. Enrofloxacin active substance solution, Reference Drug, Test Drug 1, and Test Drug 2 were administered to Group 1, Group 2, Group 3, and Group 4, respectively. Intravenous (IV) administration was performed in Group 1 whereas Groups 2, 3, and 4 received oral gastrointestinal administration at a dose of 10 mg/kg using a probe. A blood sample of 1.0–2.0 mL was collected from the animals through *v. cutanea ulnaris* into sterile heparinized tubes before the drug administration (0.0 min) and at 0.5th, 1st, 2nd, 4th, 8th, 12th, 18th, 24th, 36th, and 48th hours after drug administration starting at 5th minute in Group 1 and 0.25th hour in other groups. Blood plasma was separated into its own fractions. Following the extraction of enrofloxacin and ciprofloxacin from plasma, measurements were made using high-performance liquid chromatography (HPLC). Danofloxacin active substance was used as an internal standard (IS) in the extraction stage. Tests were performed for the sensitivity and reproducibility of the extraction method. Accordingly, HPLC showed that the time for plasma drug concentration to reach the peak value was 11.9–12.8th minutes for enrofloxacin, 8.4–9.2th minutes for ciprofloxacin, and 10.4–11.2th minutes for danofloxacin. The sensitivity of the method was determined as 0.01 µg/mL for enrofloxacin and 0.04 µg/mL for its metabolite ciprofloxacin and the recovery value of the method was found to be 75–90% for enrofloxacin and 55–70% for ciprofloxacin. In the determination of bioequivalence, values obtained by dividing the area under the curve (AUC) of the test drug 1 and test drug 2 and plasma peak plasma concentration (C_{max}) into the reference drug (0.96 and 0.97, respectively for the AUC and 0.92 and 0.97, respectively for C_{max}) were found to be within acceptable limits (0.80–1.25).

Keywords: Bioequivalence, Enrofloxacin, Broiler Chicken.

INTRODUCTION

The use of generic drugs is reported to reduce health insurance and drug expenditures by 50% and to play a key role in the national economy (Howard, 2018). Therefore, promotion of the use of generic drugs has been made necessary due to the increase in prescription drug expenditures and costs.

In the efficiency, safety, quality control and inspection of the drugs used today, bioequivalence studies are of great importance for the physician, consumer (patient), public health, manufacturer, and international trade. Successful treatment of a disease is only possible with the accurate diagnosis and the use of appropriate drugs. The exact pharmacological effect of the drug depends on the amount of active substance it contains as well as its quality and safety. There are many preparations produced by different companies that contain the same active substance, are of the same kind, are administered through the same route, and have the same area of use. The physician or patient cannot understand which preparation is safe or quality without bioequivalence studies. Therefore, bioequivalence tests are necessary and very important (Öner, 2003).

Bioequivalence applications in veterinary preparations were started later compared to human medicines. Most countries, particularly the United States of America (USA) and the European Union (EU) countries, have started to perform bioequivalence tests in veterinary medicine since the 1990s and have introduced legal regulations in this regard (Toutain and Koritz, 1997).

In Turkey, there is no regulation prepared by the Ministry of Agriculture and Rural Affairs on the conduct of bioequivalence studies for veterinary preparations (Çelik and Birdane, 2015).

The purpose of bioequivalence studies is to demonstrate the interchangeability of two or more preparations of a similar formulation (equivalent drug) (EMEA 2001, Traş and Yazar, 2002; Soyer Sarıca and Liman, 2008). It should be paid attention that the samples are as new as possible and have the same production date while selecting the reference drug and preparations to be examined (Official Gazette, 1994). The aim of this study was to examine bioequivalences of the three preparations containing enrofloxacin licensed to be used through drinking water in the poultry in Turkey (one is licensed first, others are licensed later); to identify the similarities or differences of pharmaceutically similar/identical preparations on the basis of certain pharmacokinetic variables; and to determine whether they were superior to each other in terms of clinical treatment.

MATERIALS AND METHODS

The study was approved by Animal Experiments Local Ethics Committee Faculty of Veterinary Medicine, Erciyes University, Protocol number 017/035.

Pharmaceutical preparations, active substances and analytical solutions that were used in the study are as follows: Enrofloxacin, Ciprofloxacin, Danofloxacin active substances, Reference Drug: 100 mg/mL enrofloxacin oral suspension (susp.) 500 mL/vial, Test Drug 1: 100 mg/mL enrofloxacin oral susp. 100 mL/vial, Test Drug 2: 100 mg/mL enrofloxacin oral susp. 100 mL/vial, Citrate Buffer: 5.25 mL of citrate solution + 4.75 mL of 0.100 N NaOH (pH: 6.69); Phosphate Buffer: 2.0 mL 1 M KH2PO4 + 8.0 mL 1 M Na2HPO4 (pH: 7.38) (Gündüz 1975), 10% KOH; 0.05 M NaOH.

HPLC Parameters: Model: Surveyor, UK; Detector: Diode Array Detector (DAD; Surveyor); Column: BDS C18 (250 mmX4.6 mmX5 µm); Wavelength: 278 nm; Flow rate: 1 mL/min; Mobile phase: 1700 µL of 85% orthophosphoric acid + pure water (1 L) + triethylamine (pH was set as 3.0). 850 mL was taken from this solution and then, 150 mL of acetonitrile was added into it and mixed.

Care and Feeding of the Trial Animals: The study was conducted with 40 male broiler chickens of Ross 308 race. The animals were obtained daily. The animals were kept at 30ºC in the first week and 24–29ºC in the other weeks before they were divided into groups. They were fed with feed (22% crude protein and 3000 kcal of metabolic energy) containing no residues of drugs and pollutants for 30 days (Kaya 2006). They were ensured to receive light continuously. Feed and water were given freely during the experimental period. Light and temperature were checked.

Grouping of the Animals and Administration of the Drugs

The animals were divided into four groups (Groups 1, 2, 3, and 4) at the end of 30 days, each consisting of 10 animals. In the evening before the drug application, the feed and water of the animals were removed. Drugs were administered to animals as indicated in Table 1.

Collecting Blood Samples and Plasma Analyses

Following the administration of drugs, 1.0–2.0 mL blood sample was collected from the animals through *v. cutanea ulnaris* into heparinized tubes before the drug administration (0.0 min) and at 0.5th, 1st, 2nd, 4th, 8th, 12th, 18th, 24th, 36th, and 48th hours after drug administration starting at 5th minute in Group 1 and 0.25th hour in other groups. An hour after collecting the blood samples, the plasma was separated through centrifuge at 3000 rpm for 10 minutes. Plasmas were stored at -20°C until the drug analyses. The analyses were performed within two weeks for all samples. Extraction and measurement of enrofloxacin and ciprofloxacin from the plasma were performed in accordance with the HPFC method used by Anadon et al. (1995), which was based on the method reported by Groeneveld and Brouwers (1986).

Table 1. Groups and drugs used to determine plasma drug concentration

Groups	Enrofloxacin Dose	Route of Administration	Substance Administered
Group 1	10 mg/kg (live weight)	Intravenous	Active substance
Group 2	10 mg/kg (live weight)	Oral gastrointestinal administration	Reference Drug
Group 3	10 mg/kg (live weight)	Oral gastrointestinal administration	Test Drug 1
Group 4	10 mg/kg (live weight)	Oral gastrointestinal administration	Test Drug 2

According to this, 0.5 mL plasma was taken into conical screw-cap tubes and 50 µL danofloxacin, 0.5 mL phosphate buffer solution (pH 7.5) and 1.5 mL dichloromethane were added. The tubes were sealed and mixed gently on a shaker (at 100 rpm for 10 min) and centrifuged at 2500 rpm for 10 min. The dichloromethane part at the bottom was taken into another clean vial with an insulin injector. Before being mixed on the shaker at the same rpm and speed, 0.5 mL of phosphate buffer solution and 1.5 mL of dichloromethane were added to the part at the top. It was again centrifuged at 2500 rpm for 10 min. The dichloromethane part at the bottom was taken with an insulin injector and added to the dichloromethane, which was previously taken and placed in a clean tube. Extraction was repeated once more on the part at the top. The last dichloromethane portion was taken and combined with the others. Then, the dichloromethane tube was evaporated with nitrogen flow at 30°C. At the end of the evaporation, the extract was dissolved with 0.5 mL of 0.05 M NaOH. The extract was filtered into special bottles of HPFC using a filtered syringe and submitted to HPFC. The automated sampler was set to 278 nm wavelength and to use 20 µL from this extract. The flow rate of the mobile phase was set at 1 mL/min.

Pharmacokinetic Calculations and Statistical Analyses

The area under the plasma concentration (AUC)-time curve showed that the movement of the two drugs in the body was found to be compatible with the two-compartment open model. The calculations were made with the Pharmacokinetic Calculation (PKCALC) program, which is based on the equations reported by Shumaker (1986).

Statistical analysis was performed using "SPSS 11.0 for Windows" statistics package program. Data were presented as an arithmetic mean±standard deviation. One-way analysis of variance (ANOVA) was used for pharmacokinetic data and differences between the groups were evaluated by the Duncan test (Özdemir, 2005).

For bioequivalence tests, AUC and C_{max} values were taken into consideration. The bioequivalence of the drugs was evaluated with these criteria. ANOVA was used for non-log-transformed data assuming that AUC and C_{max} values showed normal distribution (Toutain and Koritz 1997; Hantash et al. 2008; Official Gazette, 1994).

RESULTS

Amount of Active Substance in the Pharmaceutical Preparations

There should be maximum 5% difference between the amount of active substance in Test Drugs and the amount of active substance in the Reference Drug (Kayaalp, 2008).

The Reference, Test Drug 1 and Test Drug 2 were placed directly in the autosampler. The autosampler used 20 µL of each drug. Chromatography was performed for the drugs and the amount of active substance was determined. The amounts of these active substances were found to be within ±5% limit which is important in bioequivalence studies.

Enrofloxacin, ciprofloxacin and danofloxacin working standards were placed in the autosampler and their peak times were determined (Table 2).

Table 2. Peak time of the standards

Active substance	Peak time, min
Enrofloxacin	11.9–12.8
Ciprofloxacin	8.4–9.2
Danofloxacin	10.4–11.2

Extraction was performed under the same conditions for plasma separated from the clean drug-free blood taken from the animals before drug administration. The extracts were subject to HPFC and chromatography. No peak area was observed when enrofloxacin, ciprofloxacin, and danofloxacin reached the peak value.

For the Standard Curves and Recovery Value Trials, the STUDY standard containing a mixture of enrofloxacin, ciprofloxacin, and danofloxacin at a density of 1 µg/mL was prepared. The autosampler used 20 µL from this standard. Then, linear curves were drawn with the help of the peaks and areas obtained (Figure 1). The equations of the obtained curves were calculated. Then, the active substance was added to the DRUG-FREE plasma. Following the extraction, they were placed into the autosampler. The autosampler used 20 µL from this extract. Linear curves were drawn with the help of the obtained peak areas (Figure 2). The equations of the obtained curves were calculated. By using these curves, the recovery value was calculated in percentage and it was found to be 75–90% for enrofloxacin and 55–70% for ciprofloxacin.

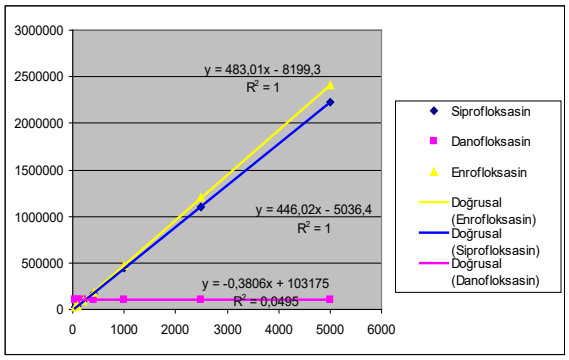

Figure 1. Standard curves of the enrofloxacin, ciprofloxacin and danofloxacin study standards drawn from the peak areas in the HPFC.

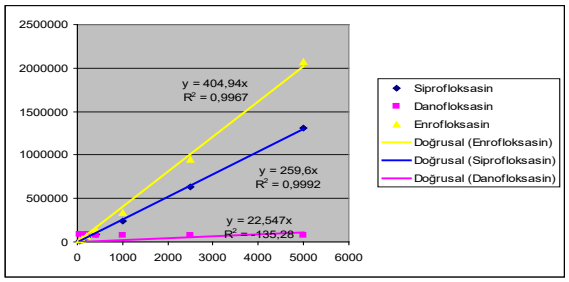

Figure 2. Standard curves of the enrofloxacin, ciprofloxacin and danofloxacin study standards drawn from the peak areas in the HPFC after adding initial standards to the drug-free plasma and extraction process.

Sensitivity of the Method: As a result of separate trials with enrofloxacin and ciprofloxacin and trials with the mixtures of these standards, the sensitivity of the method was determined to be 0.01 µg/mL for enrofloxacin and 0.04 µg/mL ciprofloxacin. It was observed that minimal values could be obtained from the lowest effective concentration (<0.5 µg/mL) of enrofloxacin in the plasma. Then, the study samples were measured. Extraction was performed in the plasma of the study samples and extracts were placed in the autosampler. The device used 20 µL from this extract.

Plasma Drug Concentrations: Figure 3 presents the logarithmic plasma drug concentration-time curves of the enrofloxacin and its metabolite ciprofloxacin measured in plasma samples following the IV administration of enrofloxacin and oral gastrointestinal administration of Reference Drug, Test Drug 1, and Test Drug 2 to animals. When the plasma drug concentration-time curve was examined following the IV administration of the drug, the distribution was found to compatible with the open two-compartment model and pharmacokinetic calculations were performed in accordance with this model. It was seen that the plasma enrofloxacin concentration reached the peak value (~ 4–5 µg/mL) at the second hour following the oral gastrointestinal administration of the reference drug, test drug 1, and test drug 2; the plasma drug concentration remained at ≥0.5 µg/mL for about 20 hours; concentration decreased below the detectable limits at 36th hour; and the plasma ciprofloxacin concentration reached the peak value (~0.11–0.39 µg/mL) at the second hour. When the reference and test drugs were evaluated together, enrofloxacin was found to produce 5–10% ciprofloxacin in the body.

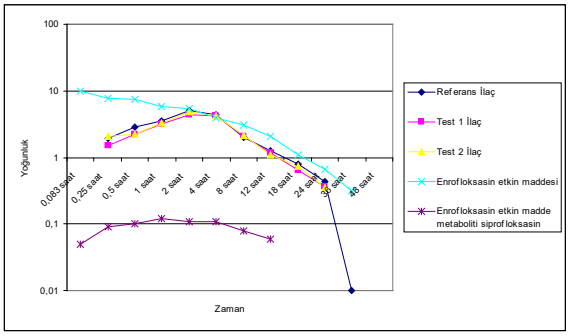

Figure 3. Logarithmic plasma drug concentration-time curve of enrofloxacin and its metabolite ciprofloxacin following the IV administration of active ingredient of enrofloxacin and logarithmic plasma drug concentration-time curves of enrofloxacin following the oral gastrointestinal administration of 10 mg/kg of the Reference Drug, Test Drug 1 and Test Drug 2.

The comparison of the values obtained from AUC, C_{max} and t_{max} logarithmic transformation in reference and test drugs showed that these were within the acceptable limits (0.80–1.25) (Table 3).

Table 3. Some pharmacokinetic variables as a result of logarithmic transformation of Reference Drug, Test Drug 1 and Test Drug 2 and bioequivalence of the test drugs

Variables	Logarithmic values			μ_{T1}/μ_R	μ_{T2}/μ_R	p- value
	Reference Drug	Test Drug 1	Test Drug 2			
AUC (µg.hr/mL)	1.66±0.01 (1.61–1.80)	1.61±0.01 (1.55–1.65)	1.62±0.02 (1.45–1.70)	0.96	0.97	0.00
C_{max} (µg/mL)	0.70±0.02 (0.56–0.77)	0.65±0.02 (0.56–0.75)	0.68±0.03 (0.44–0.80)	0.92	0.97	0.00
Acceptable Limit	0.80–1.25					

μ_{T1}/μ_R. Bioequivalence of test drug 1
μ_{T2}/μ_R. Bioequivalence of test drug 2

DISCUSSION

Despite their small number, bioequivalence studies have been conducted with some other drugs commonly used in veterinary medicine. Two different types of enrofloxacin preparations given via oral gastrointestinal administration in chickens (Posniyak et al. 2001), some sulfonamide preparations given via oral gastrointestinal administration in broilers (Altıntaş and Yarsan, 2009), two preparations containing enrofloxacin administered intramuscularly in cattle (Yılmaz, 2006), ceftiofur sodium administered intramuscularly and subcutaneously in cattle (Brown et al. 2000), two preparations containing doxycycline given via oral gastrointestinal administration in chickens (Hantash et al. 2008), carprofen administered subcutaneously and orally in dogs (Clark et al. 2003), and two different ivermectin preparations administered subcutaneously in pigs and cattle have been reported to be bioequivalent in both types (Lifschitz et al. 1999). On the other hand, Sarıca and Liman (2008) have reported that two different ciprofloxacin preparations given via oral gastrointestinal administration in broiler chickens are not bioequivalent. Sumano et al. (2001) reported that three of the four different enrofloxacin preparations given via oral gastrointestinal administration in poultry were bioequivalent while one was not.

In the study, the sensitivity of the method was determined to be 0.01 µg/mL for enrofloxacin and 0.04 µg/mL for its metabolite ciprofloxacin. In this respect, the sensitivity is higher than the findings reported by Anadon et al. (1995) (0.003 µg/mL enrofloxacin; 0.003 µg/mL ciprofloxacin; HPFC); similar to those reported by Posyniak et al. (2001) (0.02 µg/mL enrofloxacin; 0.01 µg/mL ciprofloxacin; HPFC); and lower than those reported by Şahan and Kaya (2006) (0.1 µg/mL enrofloxacin; disk-diffusion agar method).

Binding agent(s) used in the preparation of pharmaceutical formulation and content of the feed given to animals significantly affect the absorption of the drug from the digestive tract and its amount in plasma (Şahan and Kaya 2006). The sensitivity differences of the analysis methods may be attributed to the analysis methods used on the samples, the properties of the HPFC instrument and the study conditions. The sensitivity limit of the method was found to be sufficient in the present study as minimal values (50x) can be calculated from the lowest effective concentration (<0.5 µg/mL) of enrofloxacin in the plasma.

The recovery ratio was found to be 75–90% for enrofloxacin and 55–70% for ciprofloxacin in the plasma. These values were found to be similar to those determined by Posyniak et al. (2001) and Anadon et al. (1995) in plasma, and by Sumana et al. (2001) in serum (<90%, 87%, 92–97%, respectively). The recovery ratios we found were concluded to be suitable enough to render the results reliable when compared with literature data.

The bioavailability of the Reference Drug, Test Drug 1 and Test Drug 2, which were given via oral gastrointestinal administration, was found to be 66.94%, 59.28%, and 61.94%, respectively. This value was found to be similar to the findings reported by Anadon et al. (1995) (64% for the enrofloxacin active substance given via oral gastrointestinal administration), lower than the values found by Parlar and Kaya (2005) (73.44% in Group 2A [Reference Drug], 98.80% in Group 2B [Test Drug] and 74.64% in Group 2C [Test Drug] for enrofloxacin active substance administered orally through drinking water), and similar to the values of Group 2 and higher than those of Group 3 reported by Şahan and Kaya (2006) (70.43% in Group 2 and 48.67% in Group 3 for enrofloxacin active substance administered orally through drinking water). The reason for the difference between bioavailability values is thought to be due to the differences in the degree of hardness of the water in which the active substance is diluted (Sumano et al. 2001 and 2004), binding agent (Şahan and Kaya, 2006), excipients in drug formulation, and methods used.

The half-life of the enrofloxacin in the diffusion period (t1/2α) was found to be 0.03±0.00 hours in Group 1 in which the drug was administered IV, 0.46±0.10 hours for the Reference Drug, 0.71±0.08 hours for the Test Drug 1, and 0.78±0.16 hours for the Test Drug 2 which were given via oral gastrointestinal administration. The t1/2α value was found to be longer than the values found by Parlar and Kaya (2005) (0.08±0.00 hours for Group 1 [group received the drug via IV administration], 0.14±0.07 hours for Group 2A [Reference Drug] where the drug was administered through drinking water, 0.09±0.01 hours for Group 2B [Test Drug], and 0.14±0.06 hours for Group 2C [Test Drug]; disk-diffusion agar method), much shorter than those found by Kaya et al. (1996) (0.237±0.029 hours for Group 1 [group received the drug via IV administration], 2,091±0,705 hours for Group 2 [Test Drug] where the drug was given via oral gastrointestinal administration, and 3,970±1,402 hours for Group 3 [Test Drug]; disk-diffusion agar method), and shorter than the values reported by Anadon et al. (1995) (0.07±0.001 hours in the group received the drug via IV administration; 1.43±0.10 hours in the group received the drug via oral gastrointestinal administration; HPFC).

In the present study, a significant difference was observed between the group received the drug via IV administration (17.20±1.19 hours-1) and the groups received the drug via oral gastrointestinal administration (1.88±0.34 hours-1, 0.96±0.15 hours-1, and 1.58±0.78 hours-1 for reference drug, test drug 1, and test drug 2, respectively) in terms of α value ($p<0.05$).

Elimination period half-life (t1/2β) of enrofloxacin was found to be 0.61±0.20 hours in the group received the drug via IV administration and 1.69±0.14 hours for the Reference Drug, 2.09±0.30 hours for Test Drug 1 and 2.09±0.17 hours for Test Drug 2 which were given via oral gastrointestinal administration. The findings obtained in our study were similar to those found by Anadon et al. (1990) (2–3.5 hours for enrofloxacin given through drinking water; disk-diffusion agar method) whereas our time values were found to be significantly shorter than the values found by Parlar and Kaya (2005) (9.62±0.36 hours for Group 1 [group received the drug via IV administration] and 17.32±1.69 hours for Group 2A [Reference Drug], 5.33±0.21 hours for Group 2B [Test Drug], and 34.65±2.72 hours for Group 2C [Test Drug] where the drug was administered through drinking water; disk-diffusion agar method), Kaya et al. (1996) (6.079±0.056 hours for Group 1 [group received the drug via IV administration] and 14.82±4.67 hours for Group 2 [Test Drug], and 26.38±11.64 hours for Group 3 [Test Drug] where the drug was given via oral gastrointestinal administration; disk-diffusion agar method), Anadon et al. (1995) (10.29±0.45 hours in the group received the drug via IV administration and 14.23±0.46 hours in the group received the drug via oral gastrointestinal administration; HPFC), and Ovando et al. (1999) (6.99±0.48 hours in the group received the drug via IV administration; HPFC).

Mean duration of action (DoA) was found to be 16.01±1.99 hours in the group received the drug via IV administration and 8.61±0.44 hours for the reference drug, 10.10±0.63 hours for Test Drug 1, and 10.15±0.30 hours for Test Drug 2 which were given via oral gastrointestinal administration. The DoA was found to last longer in the

group receiving the drug via IV administration than in the group receiving the drug via oral administration. We believe that the reason the significantly longer DoA in the group received the drug via IV administration ($p<0.05$) is that the drug is eliminated from the body within a longer period of time when administered IV. When our findings were compared with the values reported by Kaya et al. (1996) (8.371±0.100 hours for Group 1 [IV group] and 21.44±10.51 hours for Group 2 [Test Drug] and 36.17±13.88 hours for Group 3 [Test Drug] where the drug was given via oral gastrointestinal administration; disk-diffusion agar method), DoA was found to last longer in the group where the drug was administered IV.

The AUC was found to be 69.61±3.65 µg.h/mL in the group received the drug via IV administration and 46.60±2.36 µg.h/mL for the reference drug, 41.27±1.02 µg.h/mL for Test Drug 1, and 43.12±2.37 µg.h/mL for Test Drug 2 which were given via oral gastrointestinal administration. In the present study, the AUC value of reference and test drugs administered via oral gastrointestinal route was found to be higher than the reference and test drug values found by Posyniak et al. (2001) by oral gastrointestinal administration (18.653±1.846 µg.hour/mL, 17.934±1.636 µg.hour/mL, respectively) and the value found by Kaya et al. (1996) (46.26±2.56 µg.h/mL for Group 1 [received the drug via IV administration] 18,395±2,220 µg.hr/mL for Group 2 [Test Drug] 26.91±7.97 µg.hr/mL for Group 3 [Test Drug] where the drug was given via oral gastrointestinal administration; disk-diffusion agar method), much higher than the value found by Ovando et al. (1999) (26.76±2.55 µg.hr/mL in the group received the drug via IV administration) and similar to the value reported by Parlar and Kaya (2006) (30.7±4.8 µg.hour/L for Group 2A (Reference Drug) where the drug was administered orally through drinking water, 41.3±3.4 µg.hour/L for Group 2B (Test Drug) and 31.2±3.5µg.hour/L for Group 2C (Test Drug); disk-diffusion agar method).

The time of enrofloxacin concentration to reach the peak value (t_{max}) was found to be 0.12±0.02 hours in the group received the drug via IV administration, 2.50±0.32 hours for Reference Drug, 2.75±0.36 hours for Test Drug 1, and 2.50±0.32 hours for the Test Drug 2 which were given via oral gastrointestinal administration. In this study, t_{max} value for the reference and test drugs were found to be similar to the values found by Posyniak et al. (2001) for the reference and test drugs (2.00 hours; 2.00 hours; oral gastrointestinal administration, HPFC, respectively) and the value found by Anadon et al. (1990) (2.00 hours; for enrofloxacin administered orally through drinking water; disk-diffusion agar method).

The C_{max} value of enrofloxacin was found to be 10.31±0.39 µg/mL in the group received the drug via IV administration whereas it was found to be 5.13±0.25 µg/mL for Reference Drug, 4.59±0.23 µg/mL for Test Drug 1 and 4.98±0.38 µg/mL for Test Drug 2 which were given via oral gastrointestinal administration. In the study, the C_{max} value for Reference and Test drugs was found to be significantly higher than the Reference and Test Drug values found by Posyniak et al. (2001) (0.92±1.105 µg/mL; 0.98±0.099 µg/mL, oral gastrointestinal administration, HPFC, respectively), and Anadon et al. (1990) (1.4 µg/mL; for enrofloxacin administered orally through drinking water; disk-diffusion agar method). This is thought to be due to the fact that the amount of drug entering the body in this study is higher than other studies and that the analysis methods used are different.

When the reference and test drugs are analyzed and examined in terms of pharmacokinetic variables such as AUC, C_{max}, and t_{max} and when these values are found to be within the range of bioequivalence acceptance limits (0.80–1.25 or 80–125%), the drugs can be accepted as bioequivalent (Kayaalp, 2008). The values obtained by dividing the t_{max} values of test drugs 1 and 2 (2.75±0.36 and 2.50±0.32 hours, respectively), for which logarithmic transformation could not be performed as it is a time-dependent parameter, by the Tmax value of the Reference Drug (2.50±0.32 hours) value (µT1/µR and µT2/µR) were found to be 1.00 and 1.00, respectively.

The AUC, C_{max}, and t_{max} values of the reference and test drugs were found to be within the acceptable limits (0.80–1.25) for bioequivalence. The bioequivalence limit values for Test 1 and Test 2 Drugs can be expressed as $0.80<\mu T1/\mu R<1.25$ and $0.80<\mu T2/\mu R<1.25$, respectively (Altıntaş and Yarsan 2009). Our findings have shown that all three drugs are bioequivalent and can be used interchangeably. The pharmacokinetic criteria (AUC, C_{max}, and t_{max}) evaluation of the Reference and Test Drug preparations containing enrofloxacin, which were administered via oral gastrointestinal route at the recommended doses, has shown that the drugs are bioequivalent and can be used interchangeably.

A generic drug is 20–80% cheaper than the price of the original drug. According to the data from the Pharmaceutical Manufacturers Association of Turkey (IEIS, 2009), Turkey, which is the world's 13th largest pharmaceutical market, has saved 3.8 billion Turkish liras the last five years through the use of generic drugs. Today, the promotion of the use of generic products has become government policy in countries wishing to reduce pharmaceutical costs. Economically strong countries have promoted the use of generic drugs to achieve a healthy balance between the brand-name and generic drugs and thus, they have achieved significant savings in health expenditures.

In particular, preparations containing enrofloxacin need to be used consciously since they are expensive. This study has proved that two commercial drugs containing enrofloxacin, which are manufactured by different companies, can be used interchangeably. We believe that our study will shed light on the bioequivalence studies to be performed on other drugs. In accordance with the professional awareness and responsibilities, we hope that our colleagues will make every effort to perform bioequivalence studies in medicines for animal use as well as in medicines for human use, to promote and generalize the use of generic drugs, and to raise the awareness of the owners in this regard.

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