



# Bioequivalence of a Preparation of Tulathromycin in Cattle

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## Research Article

Volume 6 Issue 1

Received Date: January 28, 2022

Published Date: February 08, 2022

DOI: 10.23880/beba-16000158

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## Abstract

Testing the quality of antibacterial pharmaceutical preparations that can be regarded as generic is of utmost importance to ensure sustainable medical practices. Hence, comparative pharmacokinetics studies become necessary. In this trial, a new pharmaceutical preparation of tulathromycin (TTM) was submitted to a bioequivalence test, taking the reference preparation of this macrolide derivative as control i.e., the commercial preparation of TTM containing 100 mg of the drug Pisadrax<sup>®</sup>, manufactured by *PiSA Agropacuaria S.A. de C.V.* and the reference brand of TTM Draxxin<sup>®</sup> (Zoetis, Mexico). Twenty-four young steers Charolais/Brahman randomly divided into two groups of twelve animals each, received a single injection of 2.5 mg/kg subcutaneously of either preparation. A validated HPLC-masses analytical technique of plasma concentrations of tulathromycin was used to determine TTM plasma concentrations at fixed intervals for 240 h. The key pharmacokinetic parameters were obtained by compartmental and non-compartmental analysis. Results show that the experimental preparation of tulathromycin (Pisadrax<sup>®</sup>) can be regarded as bioequivalent to the reference one (Draxxin<sup>®</sup>) in steers, given that AUC<sub>0-240</sub>, MRT, and K<sub>1/2el</sub> values from both preparations resulted statistically indistinguishable and with confidence interval > 95%.

**Keywords:** Tulathromycin; Bioequivalence; Steers; Pharmacokinetics; Generic

**Abbreviations:** BE: Bioequivalence; AUC: Area under the Concentration Curve; TTM: Tula Thro Mycin; BRD: Bovine Respiratory Disease; MIC: Minimum Inhibitory Concentration; UNAM: National Autonomous University of Mexico; ESI: Electrospray Ionization Detector; LOQ: Limit of Quantification; LOD: Limit of Detection.

## Introduction

The determination of the bioequivalence (BE) of veterinary drug formulations has been set as a goal in most regulatory agencies worldwide [1]. Yet, BE studies are costly and require close monitoring from regulatory officials to

ensure their adequacy for a given active principle. The compulsory implementation of BE studies awaits completion in most Latin-American countries. For a new drug-preparation to be considered bioequivalent (BE), its plasma drug-concentrations and the key pharmacokinetic values must be statistically similar to the pioneer or reference brand i.e., maximum plasma concentration ( $C_{max}$ ); area under the concentration curve vs. time (AUC), and elimination half-life ( $T_{1/2el}$ ), having administered both drug-preparations, through the same route and at the same dose [2,3]. The degree of similarity between two plasma concentration profiles to be considered BE is when the confidence interval for untransformed data should lay within a range of 80–120%,

and for logarithmically transformed data, the confidence interval is generally 80–125% [4,5]. Attainment of these targets is not always a straightforward goal and this adds considerable investment of the pharmaceutical company sponsoring the new drug preparation, and this is particularly so for the long-acting pharmaceutical preparations such as tulathromycin (TTM).

This macrolide antibiotic is used to treat bovine respiratory disease (BRD) in cattle associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* [6,7]. It is a triamilide antibiotic that exists as an equilibrium mixture of two isomeric forms, tulathromycin A (90%) and B (10%) [7]. Individual treatment of BRD-affected steers with TTM is effective, but metaphylactic treatment has been postulated as a more rational approach to control BRD because, from the herd's health perspective, significantly more TTM-treated cattle remained healthy throughout their production cycle as compared to tilmicosin-treated, or saline-treated cattle, in commercial herds [7]. Furthermore, a meta-analysis carried out with randomized clinical trials reporting treatment with TTM or tilmicosin resulted in an approximately 50% reduction in the risk of treatment or re-treatment for BRD, compared with those animals treated with tilmicosin [8].

Also, a single dose of TTM resulted effective for the treatment infectious bovine keratoconjunctivitis [9]. As a concentration-dependent antibacterial drug it is reasonable to assume that part of the referred efficacy is due to its prolonged stay in the body [7-10]. Hence, the need to carry out BE for preparations containing TTM. Another reason is to control bacterial resistance. A recent study showed that the administration of TTM induced significant changes in the nasopharyngeal and fecal microbiota, supporting an increase in the relative abundance of antibiotic resistance determinants [11].

Hence, verification of the existence of BE and consequently the maintenance of the pharmacokinetics/pharmacodynamics (PK/PD) ratios for TTM should be warranted i.e, that the plasma concentrations of this antibacterial drug are maintained above the minimum inhibitory concentration (MIC) values required for the targeted pathogens for the longest possible time between dosing intervals ( $T > MIC$ ) [12,13].

Motivated by the therapeutic success of the reference preparation of tulathromycin, a new pharmaceutical preparation of this macrolide drug has been made available in parts of the Latin-American market, and considering the importance of this drug in the cattle industry, a study was carried out to evaluate whether or not the pretending generic drug (Pisadrax<sup>®</sup>) is BE to the reference brand, Draxxin<sup>®</sup>.

## Material and Methods

### Chemicals and Reagents Drug-preparations

**Animals and housing:** All study procedures and animal care activities were carried out following the Institutional Committee for Research, Care, and Use of Experimental Animals of the National Autonomous University of Mexico (UNAM), per Official Mexican Regulation NOM-062- ZOO-1999 [14].

### Experimental Design

The sample size for this BE study was set at twelve young Charolais/Brahman steers per group based on GPower<sup>®</sup> software [15], to obtain a 0.95 test-power. Steers were randomly assigned into either the reference preparation and the experimental group with mean weights of  $357 \pm 12$  and  $368 \pm 14$ , respectively. In the former, a single injection of tulathromycin as in Draxxin<sup>®</sup> (Zoetis, Mexico) (DRAX group) at a dose of 2.5 mg/kg was injected SC in the lateral view of the neck, utilizing 18 gauge needles 2.5 cm long and a mean volume of 8.7 mL [16]. Similarly, steers from the experimental group were injected with the preparation of tulathromycin manufactured by *PiSA Agropecuaria S.A. de C.V.*, Mexico (Pisadrax<sup>®</sup>) (PISADRAX group).

Assisted by technicians, blood samples were taken by jugular puncture, collecting 8 mL in Vacutainer tubes with heparin as an additive, at the following times: just before injection, and at 15 min, 1, 2, 4, 8, 12, 24 h the first day and then every 24 h until day 10 (240 h), complying, as far as therapeutic plasma concentrations of the drug are concerned, with Nowakowski, et al. [17] and considering the expected concentration profile vs. time, proposed by EMA 2004 [18].

**Sample treatment:** Plasma was recovered from blood samples after centrifuging them (4° C; 3500 rpm/ 5 min) and immediately stored and fully identified by freezing at -20° C until analyzed.

### Analytical Chemistry

The analytical method was adapted from the one reported by Scheuch, et al. [19] as briefly described below.

**Sample preparation:** Roxithromycin was added as an internal standard to 0.25 mL aliquots of either the study plasma samples, the TTM-primed plasma samples utilized for the calibration curve, and to the quality control samples. The analytical markers were extracted from the plasma through protein precipitation with 1 mL of acetonitrile. Then, the organic phase was separated and evaporated to dryness under a stream of nitrogen at -40°C. The residue was re-dissolved with 500 µL of a solution of 45:45:10 (v/v)

acetonitrile: water ammonium hydroxide solution. From this solution, 10  $\mu$ L was injected into the chromatographic/mass-spectrometry apparatus.

**Chromatographic system:** The liquid chromatography system with mass spectrometry (LC-MS/MS) consisted of a Waters-Acquity UPLC chromatograph and a Waters TQD-Electrospray Ionization Detector (ESI), mass spectrometer. The chromatographic separation was carried out with an Acquity UPLC BEH C18 column, using as mobile phase 10 mM ammonium formate with 0.1% formic acid (v/v) in acetonitrile, with a run time of 3 min and a flow of 0.3 ml/min. The mass spectrometer was used in Multiple Reaction Mode (MRM) of positive ions with a transition of 806.68-576.96 m/z for TTM and 837.71- 678.96 m/z for roxithromycin.

The validation study was performed in terms of linearity, specificity, selectivity, accuracy, precision (repeatability and within-laboratory reproducibility), stability, matrix effect, and robustness. The decision limit (CCa), detection capability (CCb), the limit of detection (LOD), and limit of quantification (LOQ) were also estimated as shown in the results section.

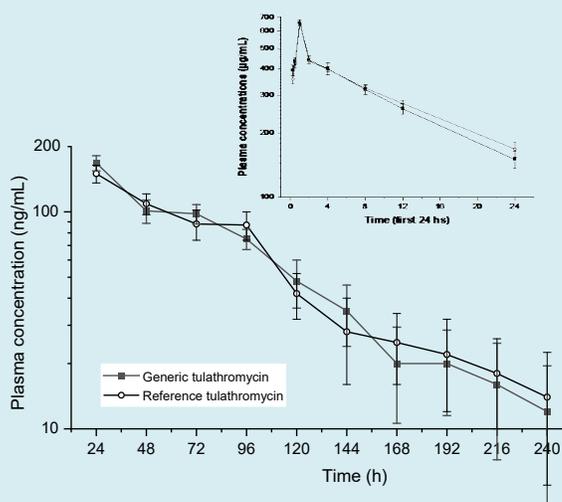
**Pharmacokinetics and statistical analysis:** Data was run through compartmental and non-compartmental models using the programs: PKAnalyst (Micromath Scientific Software, SLM, USA), Phoenix WinNonlin (Certara, USA), and using Origin Pro<sup>®</sup> (MA, USA) for graphing. Data were indistinguishable considering either model. Pharmacokinetic parameters obtained were:  $AUC_{0-240}$  ( $\mu$ g/mL/h) = area under the concentration vs time curve;  $AUMC$  ( $\mu$ g/mL/h) = area under the moment curve;  $AUC_{0-\infty}$  ( $\mu$ g/mL/h) = area under the curve extrapolated to infinity;  $K_{1/2el}$  (h) = elimination

constant;  $C_{max}$  ( $\mu$ g / mL) = maximum plasma concentration;  $T_{max}$  (h) = time to achieve  $C_{max}$ ;  $MRT$  (h) = mean residence time;  $Fr$  (%) = relative bioavailability =  $AUC_{0-240}$  of Pisadrax /  $AUC_{0-240}$  Drax x 100. The area under the concentration-time curve ( $AUC_{0-240}$ ), area under the moment curve (AUMC), and  $AUC_{0-\infty}$  were calculated using the trapezoidal rule, with extrapolation to infinity using  $C_{min}/\lambda$ , where  $C_{min}$  is the plasma concentration at the last measurable time-point.  $AUMC/AUC$  resulted in the mean residence time (MRT). The apparent volume of distribution for  $AUC_{0-240}$  ( $Vd_{AUC}$ ) was calculated as  $Vd_{AUC} = \text{total dose} / Vd_{AUC0-240} \cdot \lambda$ , and the total body clearance ( $Cl_B$ ) was calculated as  $Cl_B = Vd_{AUC} \cdot \lambda$ .

After a washout period of 21 days, a crossover maneuver was carried out, following the same conditions as above. Pharmacokinetic parameters were evaluated utilizing ANOVA and Bonferroni t-test using JMP software. The tested preparation (Pisadrax<sup>®</sup>) was considered bioequivalent if  $AUC_{0-240}$ ,  $K_{1/2el}$ , and  $C_{MAX}$  did not vary more than 20% as compared with the reference preparation (Draxxin<sup>®</sup>, Zoetis México; group Drax), and with a CI > 90%.

## Results

The implemented method presented an average recovery rate of 78.35 % (range 70.45 -89.83 %), with a linearity between 20.0 and 1200.0 ng/mL ( $r^2 > 0.988$ ), with a detection limit of 5.0 ng/mL, a quantification limit of 20.0 ng/mL and an intra-assay and inter-assay error of -12.12 and 13.5 % respectively. (Figure 1) shows mean  $\pm$  1SD plasma concentrations of tulathromycin for both groups studied, and up to 240 h post-injection.



**Figure 1:** Mean  $\pm$  1 SD of plasma concentration vs. time of tulathromycin obtained after the subcutaneous administration of 2.5 mg/kg of both Draxxin<sup>®</sup> the reference preparation and Pisadrax<sup>®</sup> the generic brand in Charolais/Brahman steers. A crossover figure is not presented to avoid redundancy as values were almost indistinguishable.

Presenting tulathromycin plasma profiles during the crossover phase was avoided as their remarkable similarity with the first one, was considered redundant. Pharmacokinetic data are summarized in (Table 1 & Table 2) shows the statistical comparison between the

pharmacokinetic parameters obtained for Draxxin® and the experimental preparation Pisadrax® by t-tests, with untransformed data. For  $T_{max}$ ,  $K_{1/2el}$ , and MRT, the comparison was made through the Wilcoxon test.

Parameter	Draxxin®	Pisadrax®
$C_{max}$ (ng/mL)	695 ± 18	688 ± 21
$T_{max}$ (h)	1.8 ± 1.52	1.4 ± 1.31
$AUC_{0-240}$ (h·ng/mL)	78,567 ± 2307	77,979 ± 2125
$AUC_{0-\infty}$ (h·ng/mL)	84,597 ± 2334	85,143 ± 2111
AUMC (h·ng/mL)	82456 ± 1687	82354 ± 1452
$\lambda_z$ (h <sup>-1</sup> )	0.0034 ± 0.0003	0.0034 ± 0.0004
$T_{1/2\lambda}$ (h)	152 ± 10.88	153.7 ± 13.62
$MRT_{0-240}$ (h)	167 ± 7.25	170 ± 11.79
$Vd_{AUC}$ (L/kg)	3.75 ± 0.29	3.70 ± 0.19
$Cl_B$ (mL/kg/h)	0.11 ± 0.03	0.12 ± 0.04

**Table 1:** Mean ± 1 SD pharmacokinetic parameters of tulathromycin obtained from non-compartment analysis of plasma concentration vs. time of tulathromycin after the subcutaneous administration of 2.5 mg/kg of either Draxxin® or Pisadrax® in Charolais/Brahman steers.

$\eta$  = 12 steers per group.

$C_{max}$ , maximum plasma concentration;  $T_{max}$ , time to reach  $C_{max}$ ;  $AUC_{0-240}$ , area under the plasma concentrations versus time curve from 0 to 240 h;  $AUC_{0-\infty}$ , area under the serum concentrations versus time curve from 0 to infinity; AUMC, area under the moment curve;  $\lambda_z$ , the slope of the elimination phase;  $T_{1/2\lambda}$ , elimination half-life;  $MRT_{0-240}$ , mean residence time from 0-240 h;  $Vd_{AUC}$ , apparent volume of distribution as calculated for AUC, after non-intravenous administration;  $Cl_B$ , Total clearance of the drug from the body after SC administration.

Parameter	t value	P value	Significance	Difference	95% Inferior limit	95% Upper limit
$C_{max}$ (ng/mL)	-1.27	0.233	NS	-173.79	-478.59	131
$T_{max}$ (h)	15	0.684	NS	NA	NA	NA
$AUC_{0-240}$ (h·ng/mL)	-0.15	0.885	NS	-228.78	-3 671.28	3 213.72
$AUC_{0-\infty}$ (h·ng/mL)	-0.08	0.937	NS	-156.03	-4 456.36	4 144.29
AUMC (h·ng/mL)	-0.09	0.94	NS	-201.5	-4 212.35	3 895.50
$\lambda_z$ (h <sup>-1</sup> )	0.62	0.549	NS	0	0	0.01
$T_{1/2\lambda}$ (h)	20	0.81	NS	NA	NA	NA
$MRT_{0-240}$ (h)	7	0.093	NS	NA	NA	NA
$Vd_{AUC}$ (L/kg)	0.03	0.978	NS	0.05	-3.82	3.91
$Cl_B$ (L/h)	-0.07	0.944	NS	0	-0.05	0.05

**Table 2:** Statistical values obtained after Student t-tests, non-parametric Wilcoxon test, and confidence intervals when comparing pharmacokinetic parameters obtained for tulathromycin after a single SC injection of Draxxin® or Pisadrax® in Charolais/Brahman steers at a dose of 2.5 mg/kg.

$\eta$  = 12 steers per group.

$C_{max}$ , maximum plasma concentration;  $T_{max}$ , time to reach  $C_{max}$ ;  $AUC_{0-240}$ , area under the plasma concentrations versus time curve from 0 to 240 h;  $AUC_{0-\infty}$ , area under the serum concentrations versus time curve from 0 to infinity; AUMC, area under the moment curve;  $\lambda_z$ , the slope of the elimination phase;  $T_{1/2\lambda}$ , elimination half-life;  $MRT_{0-240}$ , mean residence time from 0-240 h;  $Vd_{AUC}$ , apparent volume of distribution as calculated for AUC, after non-intravenous administration;  $Cl_B$ , Total clearance of the drug from the body after SC administration.

NS = not statistically significant; NA = does not apply

Based on the serum profiles of both preparations of tulathromycin, and their pharmacokinetic data obtained, it is possible to conclude that the preparations tested can be regarded as having statistically indistinguishable key pharmacokinetic parameters in cattle i.e.,  $AUC_{0-240}$ , MRT, and  $K^{1/2}_{el}$ , with confidence intervals higher than 95%, and is therefore bioequivalent.

## Discussion

Antimicrobial resistance has the potential to affect almost all sustainable production goals in food-producing animals. Among the many factors that are causing this problem, the poor quality of antibacterial pharmaceutical preparations often used in veterinary medicine, contributes in a significant manner, to heighten bacterial resistance [20]. Apart from good manufacturing practices, pharmacovigilance, and regulatory constraints to use antibacterial drugs, bioequivalence studies are very important if properly manufacture pharmaceutical preparations are going to enter the food-producing market. However, these tests can be very costly, and carry a high financial risk for manufacturers as often the results obtained turn out to be insufficient to consider a pharmaceutical preparation a truly generic treatment alternative. Hence demonstration of BE must be part of the registration file submitted to a given regulatory agency. This is usually not requested from manufacturers in most Latin American countries. Also, an important methodological issue is that BE studies must include an ideal number of participating animals according to the species and that a rational number of blood samples is to be obtained. In this context statistical power of the test should always be part of the research protocol. In this context, G\*Power (version 3.1.9.7) is a tool to compute statistical power analyses for many different t-tests, F tests,  $\chi^2$  tests, z tests, and others [15]. The statistical power of this test showed to be 0.95. The crossover procedure including the number of sampling times and animals converted the power of the test to 0.99. In this study, the plasma concentration profiles of tulathromycin and the PK data derived indicate that the experimental preparation of TTM in test (Pisadrax®) was statistically indistinguishable from the reference one when injected in cattle.  $C_{max}$  values of  $695 \pm 18$  ng/mL and  $688 \pm 21$  ng/mL for the reference and the generic preparation were not statistically different beyond the 20%, the value required to declare non-bioequivalent a given drug preparation (CI = 98%). However, if TTM is studied as a time-dependent antibacterial drug [10-12,21,22]. The absence of differences in the  $C_{max}$  would have been inconsequential, and also  $T_{max}$  would have been important if differences between the experimental and the reference preparations would have been considerable. In this context, it is safe to state that it is unlikely that  $C_{max}$  and  $T_{max}$  could have been substantially different if the number of blood samples during the first hours of the sampling period

would have been more numerous. This was also the case for  $T^{1/2}\lambda$  as a lack of statistically significant differences between Draxxin® and Pisadrax® was also ( $P > 0.8$ ), and is unlikely that increasing the number of sampling times could reveal an important difference. It is also important to underline that apart from some minor differences in the results obtained when comparing the results here obtained with other studies on the pharmacokinetics of TTM in bovine species [17, 23-25], parameters found in this study comply well with them. Differences are understandable if methodological differences are considered i.e., age of the experimental subjects, pharmacokinetics software, analytical chemistry, to mention a few.

Considering that the relative bioavailability of the generic brand and its MRT values were very similar to the corresponding ones observed in the reference preparation (see Table 3), the PK/PD ratio of  $T \geq CMI$  would be also indistinguishable. This is of utmost importance to warrant the clinical efficacy of tulathromycin [12,26]. Thus, given the  $K^{1/2}_{el}$ ,  $AUC_{0-240}$ , and MRT differences observed in this trial between the reference preparation and the generic product, it is feasible to conclude that the preparations tested can be declared BE. Furthermore, because BE can be seen as the absence of a significant difference in the rate and extent to which the active ingredient reaches either the bloodstream or tissues in a given pharmaceutical equivalent(s), clinical efficacy is likely to be comparable [27]. Yet, this latter observation requires further characterization, as in rare occasions molecular differences, such as chirality of the active principle can affect its pharmacodynamics [28,29].

Susceptibility testing of tulathromycin to find out breakpoints for field isolates indicate that most important respiratory pathogens exhibit  $MIC_{50}$  values that range from 0.125 to 4  $\mu\text{g/mL}$  [22]. Given the large  $VD_{AUC}$  found in this trial and by other authors [17], plus its immunomodulatory and anti-inflammatory actions [30] it can be assumed that drug concentrations at the target site are adequate, as meta-analysis has shown for clinical efficacy [26].

Concluding, the study of BE here presented between the reference preparation of TTM (Draxxin®) and the experimental preparation (Pisadrax®) showed absence of statistically significant differences in the key PK values, and predictably in their key PK/PD ratio ( $T \geq CMI$ ), with a power of the test  $> 0.95$ .

## Acknowledgements

This study was supported by a grant from PAPIIT IT200117, National Autonomous University of Mexico, and CONACyT, National Problems 203.

## Conflict of Interest

The authors confirm that they do not have any conflict of interest.

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