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## Pharmacokinetics and Bioequivalence of Doxycycline (Providox<sup>®</sup> and Doxyvet 0-50 S<sup>®</sup>) Oral Powder Formulations in Chickens

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**Abstract:** A bioequivalence and pharmacokinetics profiles of two doxycycline powder formulations (Providox<sup>®</sup> and Doxyvet 0-50 S<sup>®</sup>) were compared in 24 healthy chickens following administration of a single oral dose (20 mg/kg bw). Serial blood samples were drawn at 10 points after administration to determine doxycycline concentrations in chicken plasma by HPLC/UV. The pharmacokinetics parameters; area under plasma concentration-time curve (AUC<sub>0-24</sub>), maximum plasma concentration (C<sub>max</sub>) were determined for both formulations. The average means of AUC<sub>0-24</sub> and C<sub>max</sub> for Providox<sup>®</sup> and Doxyvet 0-50 S<sup>®</sup> were very close (62.32 ± 3.34 and 57.55 ± 4.66 µg.h/ml and 5.36 ± 0.26 and 5.08 ± 0.25 µg/ml, respectively) with no significant differences based on ANOVA. The 90% confidence intervals of the parameters AUC<sub>0-24</sub> and C<sub>max</sub> between two formulations were within the range 80 to 125 % of bioequivalence according to US FDA regulation. The relative bioavailability of Providox<sup>®</sup> compared to Doxyvet 0-50 S<sup>®</sup> was 108.24%. Therefore, the Providox<sup>®</sup> and Doxyvet 0 - 50 S<sup>®</sup> were considered to be bioequivalent.

**Key words:** Bioavailability, bioequivalence, chicken, doxycycline and pharmacokinetics

### Introduction

Doxycycline is a semi-synthetic bacteriostatic tetracycline and a broad-spectrum antibiotic against Gram-negative and Gram-positive aerobic and anaerobic bacteria, Rickettsiae, Chlamydiae, Mycoplasmas and some protozoa (Jha *et al.*, 1989; Prats *et al.*, 2005). Pharmacokinetics properties of doxycycline is superior than older tetracycline; in terms of higher lipid solubility, complete absorption, better tissue distribution, longer elimination half-life and lower affinity for calcium (Riond and Riviere, 1990; Goren *et al.*, 1998). The *in vitro* antimicrobial activity of doxycycline is more effective than other tetracycline for the treatment for respiratory, urinary and gastrointestinal tract diseases (Croubles *et al.*, 1998; Abd El-Aty *et al.*, 2004).

The bioavailability and bioequivalence studies play an important role in determining therapeutic efficacy to register the generic drug products according to the Food and Drug Administration (FDA) regulations (Chen *et al.*, 2001). Bioavailability is defined as the rate and extent to which an active drug ingredient is absorbed and becomes available at the site of drug of action (Martinez and Riviere, 1993). In case of Bioequivalence it is defined as statistically equivalent bioavailability between two products at the same molar dose of the therapeutic moiety under similar experimental conditions (Chen *et al.*, 2001; Toutain and Bousquet-Melou, 2004). The drug products are said to be bioequivalent if they are pharmaceutical equivalents or pharmaceutical alternatives and if their rate and extent of absorption do not show a significant differences statistically according

to the FDA regulations (Chen *et al.*, 2001).

The aim of this study was to evaluate bioequivalence of two different doxycycline powder formulations after oral administration of a single dose in chickens.

### Materials and Methods

**Drugs:** Two commercial products of doxycycline hydrochloride (hyclate) were compared. Providox<sup>®</sup> (doxycycline hydrochloride powder (200 mg/g), Provimi Jordan, Amman, Jordan) and Doxyvet 0-50 S<sup>®</sup> (doxycycline hydrochloride powder, (500 mg/g), V.M.D, Arendonk, Belgium) were used. Working doxycycline solutions of each product contained 10 mg/ml of distilled water.

**Animals:** Twenty four broiler chickens, 43-45 days old and weighing 1.7-2.1 kg, were used in this study. The animals were from Provimi Jordan research farm (Madaba, Jordan). The animals were monitored for 2 weeks for any apparent clinical signs before administration of drug. The chickens had free access to water and feed and the feed was free from antibacterial drugs. Each chicken was fasted the night before the experiment.

**Experimental design:** The chickens were allocated into two equal groups (12 chickens/group) in a parallel design. Chickens of groups 1 and 2 were given a single oral dose of Providox<sup>®</sup> and Doxyvet0-50 S<sup>®</sup>, respectively, at a dose level of 20 mg/kg body weight. This dose was based on the manufacturer's approved daily dose. The blood samples (1-1.5 ml) were drawn up to 10 times at

## Hantash *et al.*: Bioequivalence of doxycycline in chickens

Table 1: Doxycycline plasma concentrations ( $\mu\text{g/ml}$ ) in chickens after oral administration at a dosage of 20 mg/kg bw. Values are mean  $\pm$  SE (n = 12).

Time post administration (h)	Plasma concentration ( $\mu\text{g/ml}$ )	
	Provido x®	Doxyvet 0-50 S®
0.25	0.82 $\pm$ 0.05	1.12 $\pm$ 0.23
0.5	1.31 $\pm$ 0.14	1.82 $\pm$ 0.24
1.0	2.39 $\pm$ 0.16	2.02 $\pm$ 0.17
2.0	3.71 $\pm$ 0.22	3.04 $\pm$ 0.31
4.0	5.36 $\pm$ 0.52	5.08 $\pm$ 0.49
8.0	2.96 $\pm$ 0.30	3.51 $\pm$ 0.19
12.0	2.23 $\pm$ 0.29	2.30 $\pm$ 0.24
24.0	1.34 $\pm$ 0.17	1.28 $\pm$ 0.11

0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours after administration. Blood samples were collected from the brachial veins or other veins into heparinized tubes. The samples were centrifuged directly at 1000g for 5 minutes and then the plasma was harvested and stored at  $-20^{\circ}\text{C}$  until analysis.

**Drug analysis:** The plasma concentrations of doxycycline were measured using HPLC method as described previously (Axisa *et al.*, 2000). All the solvents used were of HPLC grade. The HPLC system consisted of a pump (LC-20AD) with UV-vis detector (SPD-M20A), solvent degasser (DGV-20A5) and Shimadzu LC-solution software (Ver 6.12 SP4) (Shimadzu, Japan). Chromatographic separation was performed using a Purospher Star RP-18e (5  $\mu\text{m}$ , 125 mm  $\times$  4.6 mm) column (Merck, Germany) with an isocratic mobile phase acetonitrile: methanol: 0.15% trifluoroacetic acid (23 : 25 : 52 v/v/v). The mobile phase was filtered through a 0.45  $\mu\text{m}$  membrane and degassed. The mobile phase was eluted at a flow rate of 1.5 ml/min and detected at a UV wavelength of 347 nm. A standard calibration curve was prepared by adding 200  $\mu\text{l}$  of doxycycline (1 mg/ml in water) to 800  $\mu\text{l}$  of antibacterial-free chicken plasma. This was further diluted into antibacterial-free chicken plasma to produce solutions at concentrations of 0.1, 0.5, 1, 5 and 10  $\mu\text{g/ml}$ . The peak areas were achieved by the measurement of peak area ratios using integration peak program (LC-solution software; Shimadzu, Japan). The standard curve of doxycycline in plasma which was linear at the doxycycline concentrations of 0.1 to 10 mg/ml ( $R^2=0.998$ ). The limit of quantification for doxycycline was 0.1  $\mu\text{g/ml}$ .

**Pharmacokinetics and statistical analysis:** The pharmacokinetics analysis of the data was performed using non-compartmental analysis based on the statistical moment theory (SMT) according to the method described by Gibaldi and Perrier (1982), with the help of the WinNonLin noncompartmental analysis program (version, 5.2, Pharsight, USA). The calculated parameters were: Area under plasma concentration-time curve (AUC) using linear trapezoid method; area

Table 2: Pharmacokinetics parameters of doxycycline in chickens after administration of a single oral dose of 20 mg/kg body weight. Values are mean  $\pm$  SE (n = 12).

Pharmacokinetics parameter	Providox®	Doxyvet 0-50 S®
$t_{1/2\beta}$ (h)	13.93 $\pm$ 0.84	10.06 $\pm$ 1.27
$\text{MRT}_{0-\text{inf}}$ (h)	9.36 $\pm$ 0.220	8.82 $\pm$ 0.960
$\text{Vd}_{\text{area}}/F$ (L/kg)	4.20 $\pm$ 0.270	3.60 $\pm$ 0.660
$K_{\text{el}}$ (/h)	0.04 $\pm$ 0.010	0.07 $\pm$ 0.010
$\text{Cl}_{\text{B}}$ (ml/min/kg)	0.23 $\pm$ 6.540	0.26 $\pm$ 13.91
$C_{\text{max}}$ ( $\mu\text{g/ml}$ )	5.36 $\pm$ 0.260	5.08 $\pm$ 0.250
$t_{\text{max}}$ (h)	3.60 $\pm$ 0.260	3.80 $\pm$ 0.200
$\text{AUC}_{0-1}$ ( $\mu\text{g.h/ml}$ )	62.32 $\pm$ 3.34	57.55 $\pm$ 4.66
$\text{AUC}_{0-\text{inf}}$ ( $\mu\text{g.h/ml}$ )	89.39 $\pm$ 2.95	80.02 $\pm$ 4.20

under the first moment curve (AUMC); mean residence time (MRT), where  $\text{MRT} = \text{AUMC}/\text{AUC}$ ; volume of distribution ( $\text{Vd}_{\text{area}}$ ), where  $\text{Vd}_{\text{area}} = \text{dose}/\text{AUC} (\beta)$ ; total body clearance ( $\text{Cl}_{\text{B}}$ ), where  $\text{Cl}_{\text{B}} = \text{dose}/\text{AUC}$ ; elimination rate ( $K_{\text{el}}$ ) was determined by least-square regression analysis of terminal log-linear portions of the plasma concentration-time profile ( $K_{\text{el}} = 2.303 \times \text{slop}$ ); elimination half-life ( $t_{1/2\beta}$ ), where  $t_{1/2\beta} = 0.693/K_{\text{el}}$ ; the maximum concentration ( $C_{\text{max}}$ ) and the corresponding peak time ( $t_{\text{max}}$ ) were determined by inspecting the individual drug plasma concentration-time profiles. The relative bioavailability (F) was calculated as  $(\text{AUC}_{\text{reference}}/\text{AUC}_{\text{test}}) \times 100$ . The bioequivalence of drug products were evaluated by comparing the test and reference products parameters; AUC and  $C_{\text{max}}$  values through the 90% confidence intervals test were within the range 80 to 125% according to FDA regulations (Chen *et al.*, 2001).

Statistical analysis on the pharmacokinetics parameters of doxycycline products were assessed by analysis of variance (ANOVA). The differences were considered significant when  $p < 0.05$ . All data are expressed as mean  $\pm$  SE.

## Results

The plasma concentration-time profiles of two formulations were similar through the entire study periods (Table 1 and Fig. 1). Plasma doxycycline was detected at first sampling time (15 minutes) and gradually increased and reached a peak concentration ( $C_{\text{max}}$ ) of 5.36  $\pm$  0.26 and 5.08  $\pm$  0.25  $\mu\text{g/ml}$  at 3.6  $\pm$  0.22 and 3.8  $\pm$  0.26 h for Providox® and Doxyvet0-50 S®, respectively (Table 2). Doxycycline's concentrations declined below limits of quantification at 48 hours for both formulations. The average mean of  $\text{AUC}_{0-24}$  for Providox® and Doxyvet0-50 S® was 62.32  $\pm$  3.34 and 57.55  $\pm$  4.66 ( $\mu\text{g.h}$ )/ml, respectively (Table 2). However, long elimination half-lives ( $t_{1/2\beta}$ ) (13.93  $\pm$  0.84 and 10.06  $\pm$  1.27 h), low total body clearance ( $\text{Cl}_{\text{B}}$ ) (0.23  $\pm$  6.54 and 0.26  $\pm$  13.91 ml/min/kg) and high volume of distribution ( $\text{Vd}_{\text{area}}/F$ ) (4.20  $\pm$  0.27 and 3.60  $\pm$  0.66 L/kg) were determined for Providox® and Doxyvet0-50 S®, respectively (Table 2). No significant differences were

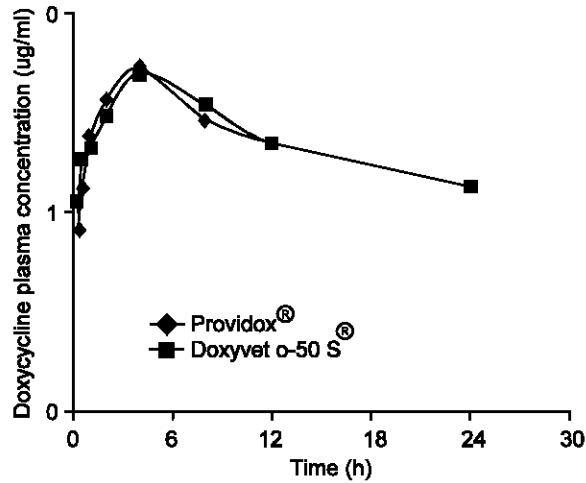


Fig. 1: Plasma concentration-time profile of doxycycline after oral administration of 20 mg/kg body weight. Values are mean  $\pm$  SE (n = 10).

found among all the tested pharmacokinetics parameters. The relative bioavailability of Providox® compared to Doxyvet0-50 S® was 108.28 % (Table 3). The 90 confidence interval ranges for  $C_{max}$  and  $AUC_{0-24}$  of Providox® compared to Doxyvet0-50 S® were 94.5 to 117.28 and 97.56 to 122.63 % respectively, (Table 3).

**Discussion**

The pharmacokinetics of doxycycline was reported in chickens following different routes of administrations (Anadon *et al.*, 1994; Laczay *et al.*, 2001; Ismail and El-Kattan, 2004). However, no studies are available on the pharmacokinetics comparisons and bioequivalence for different doxycycline powder formulations after oral administration in poultry. Therefore, the current study was designed to investigate pharmacokinetics and bioequivalence of doxycycline in broiler chickens of two powder formulations after oral administration.

Doxycycline plasma concentrations were detected at first sampling (0.25 h) with a similar  $C_{max}$  ( $5.36 \pm 0.52$  and  $5.08 \pm 0.25$  µg/ml) and  $AUC_{0-24}$  ( $62.32 \pm 3.34$  and  $57.55 \pm 4.66$  (µg.h)/ml) for Providox® and Doxyvet0-50 S®, respectively. Elimination half lives of Providox® and Doxyvet0-50 S® after a single oral administration to chickens at a dose 20 mg/kg body weight was long and reaches  $13.93 \pm 0.84$  and  $10.06 \pm 1.27$  h, respectively. The long  $t_{1/2\beta}$  is a clear characteristic of doxycycline in different species, which range from 4.2 to 16.6 h (Jha *et al.*, 1989; Anadon *et al.*, 1994; Santos *et al.*, 1996; Baert *et al.*, 2000; Laczay *et al.*, 2001). High volume of distribution ( $4.20 \pm 0.27$  and  $3.60 \pm 0.66$  L/kg) and a low total body clearance ( $0.23 \pm 6.54$  and  $0.26 \pm 13.91$  ml/min/kg) for Providox® and Doxyvet 0-50 S®, respectively; indicates that doxycycline is rapidly absorbed, widely distributed and slowly eliminated in the

Table 3: Bioequivalence results of Providox® (test product) and Doxyvet 0-50 S® (reference product).

Geometric means			
Pharmacokinetic parameter	$AUC_{0-24}$	$C_{max}$	
Test product	62.32	5.36	
Reference product	57.55	5.08	
Ratio test/reference product (%)	108.24	105.51	
90% confidence interval (CI)			
Low	97.56	94.5	
High	122.63	117.28	

body after oral administration in chickens as well as reported by Anadon *et al.* (1994), Laczay *et al.* (2001) and Ismail and El-Kattan (2004). Doxycycline peak plasma concentration for both formulations was higher than the minimum inhibitory concentrations (MICs) for *Mycoplasma gallisepticum* (0.2 µg/ml) (Takahashi and Yoshida, 1989), *Mycoplasma Pneumoniae* (< 0.5 µg/ml) (Waites *et al.*, 2003), *Staphylococcus aureus* (0.25 µg/ml) (Bryant *et al.*, 2000), *Streptococcus pneumoniae* (< 0.4 µg/ml) (Aronson, 1980) and *E. coli* (1-4 µg/ml) (Moskowitz *et al.*, 2004). However, doxycycline peak plasma concentration for both formulations was lower than the MICs for *Pseudomonas aeruginosa* (>64 µg/ml) (Toutain and Bousquet-Melou, 2004) and *Enterococcus faecalis* (8 to 32 µg/ml) (Hoelscher *et al.*, 2006). This emerge the therapeutic usefulness of doxycycline in control many susceptible bacteria.

Bioequivalence study is a test to assure the clinical efficacy of a generic versus brand drugs (Chen *et al.*, 2001). Our results showed that the ratios of mean values of two doxycycline powder products were around 100 % (Table 3) in  $AUC_{0-24}$  and  $C_{max}$  and the 90% confidence intervals of both parameters for Providox® were within the acceptable range (80-125%) (Table 3) when compared with the Doxyvet0-50 S®. Both formulations were shown to be bioequivalent in terms of rate and extent of absorption. No significant differences were observed between the pharmacokinetics parameters of the two formulations, these results were showing the bioequivalence of the two formulations were according to the criteria established by FDA (Chen *et al.*, 2001).

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Hantash *et al.*: Bioequivalence of doxycycline in chickens

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