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BIOEQUIVALENCE STUDY OF TWO ORAL SULFACLOZINE (ESB3® AND ORACLOZIN®) IN BROILER CHICKENS

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ABSTRACT

The present study was designed to assess the comparative bio-equivalence of ESB3® and Oraclozin® in healthy broiler chickens after oral administration of both products in a dose of 30 mg sulfaclozine/Kg b. wt. Twenty four broiler chickens were divided into two groups. The first group was designed to study the pharmacokinetics of ESB3®, while the 2nd group was designed to study the pharmacokinetics of Oraclozin®. Each broiler chicken in both groups was orally administered with 30 mg sulfaclozine/Kg b. wt. Blood samples were obtained from the wing vein and collected immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after a single oral administration. The disposition kinetics of ESB3® and Oraclozin® following oral administration of 30 mg sulfaclozine/Kg b. wt, revealed that the maximum blood concentration of sulfaclozine [C_{max}] were 16.21 and 15.24 µg/ml and attained at [t_{max}] of 4.06 and 4.01 hours, respectively. In conclusion: Oraclozin® is bioequivalent to ESB3® since the ratios of C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ (T/R) was 0.94, 0.92 and 0.92 respectively. These are within the bioequivalence acceptance range. Oraclozin® and ESB3® are therefore bioequivalent and interchangeable.

KEYWORDS: These are within the bioequivalence acceptance range.

INTRODUCTION

Sulphonamides were the first drugs used for systemic treatment and the prevention of bacterial infections. Sulfaclozine is an efficacious sulphonamide derivative with antibacterial

(Gram-positive and Gram-negative) and anticoccidial effects, and is commonly used for the treatment of various poultry diseases (particularly, collibacteriosis, fowl cholera and coccidiosis). Its mechanism of action is similar to that of other sulphonamides. These compounds replace para-aminobenzoic acid (PABA) in the metabolism of protozoa and bacteria, and induce their effects by inhibiting the synthesis of folic acid. Sulfaclozine is weakly absorbed from the gastrointestinal tract. Sulphonamides are subject to biotransformation in various tissues, mainly the liver, at varying degrees and through varying mechanisms (oxidation, acetylation, resolution of the ring, and binding with glucuronic, sulphuric acid, etc.). Among these mechanisms, the most important is the N4-acetylation reaction. Each sulphonamide undergoes this reaction at a varying level. The N4-acetylation reaction varies with animal species. N4-acetylated metabolites do not have any activity and are lowly soluble in water. Due to their low solubility in water, they may precipitate in the renal tubules and cause damage. Sulphonamides are excreted mainly via the kidneys, and to a limited extent, in bile and feces. Sulphonamides may have an antagonistic effect with local anaesthetics containing PABA (i.e. procaine, butacaine) and procaine penicillin G. The effects of sulphonamides are weakly antagonized by B-complex vitamins, such as nicotinamide, folic acid and choline, as well as by the precursors of these vitamins, including glutamic acid, methionine.[1-9]

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.[10] Bioavailability is defined as the rate and extent to which an active drug ingredient is absorbed and becomes available at the site of drug action. 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.[10-11] 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.[10]

The aim of this study is to evaluate bioequivalence of two oral sulfaclozine powders **(ESB3®** and **Oraclozin®**) after oral administration of a single dose in broiler chickens.

MATERIALS AND METHODS

Drugs

ESB3® was obtained from **Elanco Animal Health, USA** (it was used as reference product) and **Oraclozin®** was obtained from **Boston Company, Pharma-Right Group, Egypt (**it was used as test product). Both are formulated as water soluble powders. Each 100 gram contains 30 g sulfaclozine sodium.

Broiler Chickens and Experimental Design

Twenty four healthy broiler chickens (30 days old and weighing 1.60 – 1.85 kg) were obtained from Benha private poultry farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20˚C), and 14 hours of day light. They received a standard commercial ration free from any antibiotics before starting the experiment to insure complete clearance of any anti-bacterial substances from their bodies. Water was offered *ad-libitum*.

Bioequivalence Study

Broiler chickens were used to study the bio-equivalence of **ESB3® and Oraclozin®** after oral administration. Broiler chickens were divided into two groups. The 1st group (12 broiler chickens) was used to study the pharmacokinetics of **ESB3®**. The 2nd group (12 broiler chickens) was used to study the pharmacokinetics of **Oraclozin®**. Broiler chickens in the 1st group were administered orally (in drinking water) with **ESB3®** in a dose of 30 mg sulfaclozine/Kg b.wt, while broiler chickens in the 2nd group were administered orally with **Oraclozin®** in a dose of 30 mg sulfaclozine/Kg b. wt.

Blood Samples

Blood samples were obtained from the wing vein (1 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single oral administration (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of sulfaclozine concentration. The serum samples were stored at −20˚C until drug assay.

Analytical Procedure

Serum samples were extracted as described by.[12] Accordingly, 0.2 ml of 1 M phosphate buffer (pH 6.3) was added to 0.5 ml of the sample, and the mixture was vortexed. Subsequently, 5 ml of ethyl acetate was added, and the mixture was thoroughly mixed for 15 min by vortexing. Then it was centrifuged at 3000 rpm for 10 min. Two milliliter of the

supernatant was transferred into another tube, and was evaporated at 50 ^{o}C until dried. Subsequently, the residue was dissolved in 100 µl of methanol and 20 µl was applied to the HPLC apparatus. Analysis was performed using a HPLC apparatus at 270 nm with an UV detector and at a flow speed of 2 ml/min using a C_{18} column (250 x 4.6 mm – 5 µm) at the mobile phase (845 ml deionised water + 145 ml acetonitrile + 10 ml glacial acetic acid).[12-13] Sulfadoxin was used as an internal standard in analysis.

Prior to the analysis of the samples, the method used for the detection of serum sulfaclozine was validated. For this purpose, serum samples obtained from chicks, which were not administered any drugs, were supplemented with certain concentrations of sulfaclozine (2.5, 5.0 and 10.0 µg/ml).

Pharmacokinetics analysis

Serum concentrations of sulfaclozine versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a compartmental and non-compartmental analysis using computerized program, WinNonline 4.1 (Pharsight, USA).

The peak concentrations, C_{max} and time to peak, T_{max} were obtained from the serum concentration-time data directly. The areas under the serum concentration of sulfaclozine time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method.[14] While $AUC_{0-\infty}$ was derived from AUC_{0-24} + $AUC_{24-\infty}$, where $AUC_{24-\infty}$ = C_{24}/ß. For bioequivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the bioequivalence acceptable range at 90% confidence interval, 0.80 – 1.25 were considered for accepting the null hypothesis of bioequivalence between the reference and the test brands.[15-16]

RESULTS

The mean serum concentrations of sulfaclozine in **ESB3® and Oraclozin®** following oral administration of 30 mg sulfaclozine/Kg b.wt in broiler chickens are shown in (**Table 1** and **Figure 1**).

Table 1: Mean (X ± S.E) serum concentrations (µg/ml) of sulfaclozine in ESB3® and Oraclozin® following oral administration of 30 mg sulfaclozine/Kg b.wt in broiler chickens (n = 12).

Time post Administration (hour)	Mean serum concentration (µg/ml)	
	Group 1ESB3® (Reference)	Group 2 Oraclozin® (Test)
0.08	0.63±0.04	0.51±0.02
0.16	1.51±0.07	1.11±0.05
0.25	2.12±0.09	1.98±0.07
0.5	3.14±0.15	2.85±0.12
1	6.51±0.23	5.94±0.36
2	11.32±0.45	10.78±0.42
4	20.65±0.63	19.72±0.42
6	15.51±0.72	14.63±0.69
8	10.45±0.39	9.44±0.31
12	5.31±0.03	4.82±0.13
24	1.05±0.06	0.93±0.07

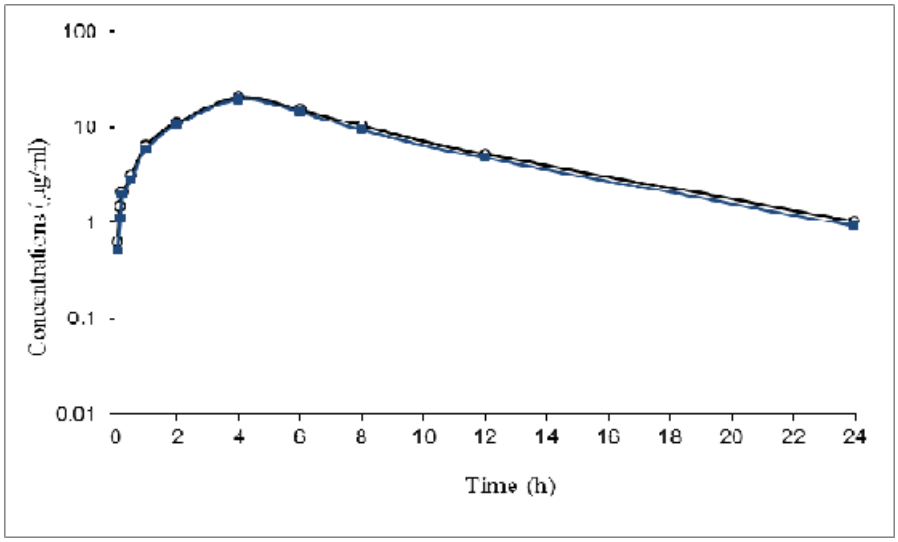

Figure 1: Semilogarthimic plot showing the serum concentrations-time profile of sulfaclozine in ESB3® (○) and Oraclozin® (■) following oral administration of 30 mg sulfaclozine/Kg b.wt in broiler chickens (n = 12).

The mean pharmacokinetic parameters of sulfaclozine in **ESB3®** and **Oraclozin®** after oral administration of 30 mg sulfaclozine/Kg b.wt. in broiler chickens are shown in (**Table 2**).

Table 2: Mean (X ± S.E) pharmacokinetic parameters of sulfaclozine in ESB3® and Oraclozin® following oral administration of 30 mg sulfaclozine/Kg b.wt in broiler chickens (n = 12).

Parameter	Unit	ESB3® (Reference)	Oraclozin®(Test)
K_{ab}	h^{-1}	0.246 ± 0.01	0.248 ± 0.01
K_{el}	h^{-1}	0.141 ± 0.001	0.143± 0.001
$t_{1/2(ab)}$	h	2.81 ± 0.01	2.78 ± 0.02
$t_{1/2(el)}$	h	4.89 ± 0.11	4.84 ± 0.10
C_{max}	µg ml^{-1}	16 .21± 0.37	15.24 ± 0.48
t_{max}	h	4.06 ± 0.29	4.01 ± 0.17
AUC	µg $ml^{-1}h^{-1}$	183.44 ± 10.15	169.82±11.63
AUMC	µg $ml^{-1}h^{-2}$	1532.87 ± 982.6	1395.40 ±102.99
MRT	h	8.35 ± 0.28	8.21 ± 0.41

k_{ab}; K_{el} absorbtion and elimination rate constant after oral administration; **$T_{1/2(ab)}$** absorbtion half life after oral administration; **$T_{1/2(el)}$** elimination half life after oral administration; **C_{max}** maximum plasma concentration; **T_{max}** time to peak plasma concentration; **AUC**; area under serum concentration-time curve; **AUMC** area under moment curve; **MRT** mean residence time.

The disposition kinetics of sulfaclozine in **ESB3®** and **Oraclozin®** following oral administration of 30 mg sulfaclozine/Kg b.wt, revealed that the maximum blood concentration [C_{max}] were 16.21 and 15.24 µg/ml and attained at [T_{max}] of 4.06 and 4.01 hours, respectively. The mean ratio of C_{max} and AUC of the reference and tested formulations were within bioequivalence range and summarized in **Table 3**. All the experimental chickens remained healthy during and after the study.

Table 3: Bioequivalence between ESB3® (reference) and Oraclozin® (test) formulations.

Bioequivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
ESB3® (reference)	16.21±0.37	176.02±8.38	183.44±10.15
Oraclozin® (test)	15.24±0.48	163.32±9.59	169.82±11.63
Point estimate	0.94	0.92	0.92
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	BE	BE	BE

BE-Bioequivalence

DISCUSSION

Research has been conducted on the pharmacokinetics of various sulphonamides in poultry. In these studies, certain kinetic parameters of sulphonamides administered by different routes have been investigated.[16-17] In a previously conducted study on the pharmacokinetics of

sulphachloropyrazine (sulfaclozine), this compound was administered to roosters[5] by only intravenous route and the influence of testosterone hormone on pharmacokinetic parameters was investigated. However, although sulfaclozine is commonly administered in drinking water or feed (indirectly intracrop) for the treatment of various microbial diseases and particularly intestinal coccidiosis in poultry, only one previous study exists on the pharmacokinetic parameters of sulfaclozine administered by both intravenous and intracrop routes in broiler chickens.[18]

The t_{max} value being long, whilst C_{max} being low, in view of the dose administered, were also considered to be in support of the interpretation of the absorption of the drug.[18] Among relevant pharmacokinetic studies conducted using the same or similar sulphonamides, in an investigation conducted by[5], in which sulphachloropyrazine (sulfaclozine) was administered at a dose of 50 mg/kg bw, the $t_{1/2b}$, and MRT values were reported as 17.32 h and 24.87 h, respectively. In the study performed by,[18] these values were determined to be higher. Furthermore,[17] upon administering 33.34 mg/kg bw sulphadiazine by oral route, reported $t_{1/2b}$ as 3.2 h. In a study in which sulphaquinoxaline (100 mg/kg bw) was administered to broiler chickens, C_{max} and T_{max} values to be 107.80 µg/ml and 5.56 h, respectively, for oral administration.[16] When orally administered to broiler chickens at a dose of 200 mg/kg bw, reported the $t_{1/2b}$ value of sulphamonomethoxine and sulphaquinoxaline in various tissues (plasma, liver, kidney, lung and muscle) to be 4.7–9.0 and 4.5–18.9 h, respectively. Concentration of sulfaclozine in serum from 5 min up to 24 h exceeds the MIC against sensitive micro-organisms.

Bioequivalence study is a test to assure the clinical efficacy of a generic versus brand drugs.[10] Bioequivalence refers to a comparison between generic formulations of a drug, or a product in which a change has been made in one or more of the ingredients or in the manufacturing process, and a reference dosage form of the same drug. This study shows that the bioequivalence ratio for mean AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} (T/R) of **Oraclozin®** versus the reference products (**ESB3®**) were 0.92, 0.92 and 0.94 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 – 1.25.[19] The two formulations of sulfaclozine oral tested in this experiment could therefore be considered bioequivalent.

CONCLUSIONS

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that **Oraclozin® (Boston Company, Pharma-Right Group, Egypt)** was bioequivalent to **ESB3® (Elanco Abimal Health, USA)** and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

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