

## Bioequivalence of Some Sulphaquinoxaline Formulations Following Oral Administration in Broiler Chickens

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**Abstract:** The present study was carried out to determine the bioequivalence of 2 different products of sulphaquinoxaline (SQ) used orally in broiler chickens. In the present study, 40 unmedicated 28 day-old chicks (Ross 308) were used. Animals were divided into 4 experimental groups, each containing 10 chicks. Sulphaquinoxaline at a level of 100 mg kg<sup>-1</sup> BW was given to Group I via intravenously and Group II, III and IV via the intracrop route. Blood samples were taken into sterilized tubes at 5, 15, 30 and 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 18, 24 and 36 h following drug administration. Plasma SQ concentrations were measured by spectrophotometer (Schimadzu, UV1601). Both drugs were distributed according to a 2-compartment open model following their administration of intravenously. The sensitivity of the extraction method for SQ was detected as 1.41 µg mL<sup>-1</sup>; the mean recovery value of the extraction procedure for SQ was detected as 90%. When compared the drugs of Group III (reference; A) and Group IV (test; B) for SQ bioequivalence; although mean AUC and C<sub>max</sub> values decreased, an increase in mean t<sub>max</sub> values was observed. Mean AUC and C<sub>max</sub> values for SQ were found to be within acceptable ranges (80-125%) when compared with mean AUC and C<sub>max</sub> values for A and B drugs. Data obtained in the present study showed that both drugs had similar bioequivalence. As a result it was concluded that both drugs could be substituted for each other as an inter-changeable drug.

**Key words:** Sulphaquinoxaline, bioequivalence, pharmacokinetic, broiler

### INTRODUCTION

An equivalent drug is one which is therapeutically equivalent to an original pharmaceutical product (reference drug) whose patent has expired. These products include basically the same drug substance. Bioequivalence tests are scientific and biologically-based tests used for comparing different preparations in similar formulations which contain the same drug substance. The purpose of the bioequivalence tests is to determine whether 2 products have the same characters in terms of effectiveness and safety of their systemic impacts. In parallel with this, they show the plasma concentrations of the products in question. In addition, these tests help determine the relative safety performance of a particular drug and so help establish a preference over alternative drugs in the same field. Bioequivalence tests, consumers, doctors, public health officials, producers and international trade also have determining roles. Bioequivalence studies are conducted on veterinary medicine drugs as well as on human drugs in the USA and EU (EMA, 2001; Posyniak *et al.*, 2001; Traş and Yazar, 2002). The essence of bioequivalence studies is to compare the bioequivalence of the drug to be tested against the reference product. The reference product is

regarded as licensed and full-dose. The bioavailability of the drug has a determining role in the evaluation of its bioequivalence. Pharmacokinetics is commonly used in the determination of bioequivalence and bioavailability. The acceptable limits at the 90% confidence interval for the area under the plasma concentration and time curve are 0.80-1.25 (80-125%). Although, this option is only available for Y<sub>max</sub> (Y<sub>max</sub>), this interval can be extended on the condition that effectiveness and safety are taken into consideration.

Sulphonamides, which are frequently used in veterinary medicine, are the synthetic derivatives of para-aminobenzenesulphonamides and are the first effective chemotherapeutic agents used systematically for the treatment and prevention of bacterial infections in humans and animals. Sulphonamids show their effects by stopping the reproduction of bacteria and preventing them from developing. Thus, the bacteria, devoid of their development process are eliminated by the defense systems of the body. They may cause fatal effects in bacteria in high-concentrations. They are particularly effective during the fast development or reproduction process of the bacteria.

Sulphaquinoxaline is a wide-spectrum drug stemming from the sulphonamid. It bears the general characteristics

of the sulphonamid group in hindering the reproduction and the development processes of bacteria. In addition, it prevents the effectiveness of dihydropteroate synthetase which catalyzes the reaction between Para-aminobenzoic acid (PABA) and dihydropterine and thereby inhibits the synthesis of folic acid. Sulphonamids, as a group, are effective on gram-positive bacteria as well as gram-negative bacteria, Rickettsia, Chlamydia and protozoa species. The most common derivatives of sulphonamid, used for treatment and prevention of coccidiosis, are sulphaquinoxalin, sulphadiazine, sulphadimetoxin, sulphadoxin, sulphamethazine, sulphamethoxazol, sulphanitran and sulphachloropirazine.

The purpose of this study is to determine the bioequivalence of sulphaquinoxalin preparations commonly used for chick diseases.

## MATERIALS AND METHODS

The study used 40 broiler chicks of the Ross 308 stock:

**A drug (reference):** Total 200 mL oral suspension containing 32 mg sulphaquinoxalin.

**B drug (Test):** Total 200 mL oral suspension containing 32 mg sulphaquinoxalin.

The chicks were kept in a poultry house at a temperature of 30°C during the first week and at a temperature of between 24-29°C in the following weeks. They were provided with an area where there was always light. The animals were given feeds which included no drugs or additives during the testing period. Their feed comprised 23 raw protein, 6 raw cellulose, 8% raw ash and 3.100 kcal kg<sup>-1</sup> metabolic energy. After 28 days, the animals were divided into 4 groups, each of 10 animals.

The animals in Group I were administered sulphaquinoxalin sodium standard equivalent to 100 mg kg<sup>-1</sup> c.a. sulphaquinoxalin through their underwing veins. Blood samples were taken at the 5, 15, 30 and 45 min and the 1st, 1.5th, 3rd, 6th, 9th, 12th, 18th and 24th h after they were administered drugs. These samples were placed in heparinized tubes (100 units of heparin in 1 mL blood). The animals in Group II were administered sulphaquinoxalin sodium standard equivalent to 100 mg kg<sup>-1</sup> c.a. sulphaquinoxalin directly through their feed. Blood samples were taken at the 15.20 and 45 min and 1st, 1.5th, 3rd, 6th, 9th, 12th, 18th, 24th and 36th h after they were administered drugs. These samples were placed in heparinized tubes (100 units of heparin in 1 mL blood). The animals in Group III were administered reference drug equivalent to 100 mg kg<sup>-1</sup> c.a. sulphaqui-

noxalin through their feed with the help of drainage. Blood samples were taken at the 15.20 and 45 min and 1st, 1.5th, 3rd, 6th, 9th, 12th, 18th, 24th and 36th h after they were administered drugs. These samples were placed in heparinized tubes (100 units of heparin in 1 mL blood). The animals in Group IV were administered Test Drug equivalent to 100 mg kg<sup>-1</sup> c.a. sulphaquinoxalin through their crops with the help of drainage. Blood samples were taken at the 15.20 and 45 min and 1st, 1, 5th, 3rd, 6th, 9th, 12th, 18th, 24th and 36th h after they were administered drugs. These samples were placed in heparinized tubes (100 units of heparin in 1 mL blood). The samples were centrifuged for 15 min at 3,000 rpm and their plasmas were extracted. They were stored at a temperature of -18°C prior to analysis. The sulphaquinoxalin concentration in plasma was spectrophotometrically determined according to the method of Hammond (1977) who based his views on Bratton and Marshall (1939).

Drug concentration and time curves of the plasmas were drawn and EAA,  $Y_{max}$ ,  $t_{max}$  values, which are necessary for bioequivalence studies, were determined. The results were statistically assessed (Wagner, 1975). The SPSS 11.0 for Windows program was used for statistical calculations.

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## RESULTS

With the help of the absorbance obtained from the standards, the standard curve of sulphaquinoxalin was drawn and the equation of the linear curve was calculated. The sensitivity of the system was determined as 1.41 µg mL<sup>-1</sup> and the recycling rate was determined as 90%. The drug concentration and time curves were examined (Fig. 1) and the regression analysis of the curve was made according to different division methods following the intravenous administration of the drug mixture. The distribution of SQ ( $r^2$ : 0.99) in the body was determined to be suitable for the 2-section outward model and pharmacokinetic parameters were calculated by taking into account this distribution (Table 1).

When Group IV (test drug) was compared to Group III (reference drug), there was a reduction in EAA,  $Y_{max}$  whereas there was an increase in  $t_{max}$  values. However, this difference between the drugs was determined to be within the acceptable limits for them to be equivalent drugs (Table 2).

Table 1: Pharmacokinetic variables of SQ administered intravenous (Group I) and intracrop (Group II, III and IV) (arithmetic average±standard deviation)

Sulphaquinoxalin (SQ)				
Variables	Group I (min-max values)	Group II (min-max values)	Group III (min-max values)	Group IV (min-max values)
$\alpha$ (hour <sup>-1</sup> )	3.586±1.278 <sup>a</sup> (2.06-5.10)	0.184±0.046 <sup>b</sup> (0.12-0.24)	0.197±0.094 <sup>b</sup> (0.10-0.35)	0.133±0.032 <sup>b</sup> (0.09-0.19)
$\beta$ (hour <sup>-1</sup> )	0.0412±0.001 <sup>a</sup> (0.04-0.04)	0.0365±0.002 <sup>bc</sup> (0.03-0.04)	0.0344±0.003 <sup>b</sup> (0.03-0.04)	0.0379±0.001 <sup>c</sup> (0.03-0.04)
$t_{1/2\alpha}$ (hour)	-	1.565±0.235 <sup>a</sup> (1.30-1.83)	1.470±0.498 <sup>a</sup> (0.45-2.1)	1.891±0.644 <sup>a</sup> (1.11-2.67)
$t_{1/2\beta}$ (hour)	16.837±0.658 <sup>a</sup> (16.19-17.91)	19.045±1.215 <sup>bc</sup> (17.84-21.26)	20.332±2.294 <sup>c</sup> (17.59-24.40)	18.278±0.547 <sup>ab</sup> (17.77-19.09)
$t_{1/2\alpha}$ (hour)	0.220±0.086 <sup>a</sup> (0.140-0.34)	4.010±1.161 <sup>b</sup> (2.92-6.03)	4.204±1.726 <sup>bc</sup> (2.00-6.73)	5.481±1.309 <sup>c</sup> (3.610-7.620)
ART (hour)	24.085±0.917 <sup>a</sup> (23.18-25.51)	28.410±1.347 <sup>b</sup> (26.01-30.24)	29.958±1.934 <sup>b</sup> (27.29-32.25)	28.437±0.979 <sup>b</sup> (26.80-29.73)
$T_{max}$ (hour)	-	6.128±0.623 <sup>a</sup> (5.36-6.75)	5.870±1.559 <sup>a</sup> (2.65-7.76)	6.810±1.585 <sup>a</sup> (4.76-8.59)
$Y_{max}$ (µg mL <sup>-1</sup> )	-	196.070±33.712 <sup>a</sup> (145.96-296.61)	226.301±19.569 <sup>a</sup> (196.16-253.49)	214.201±32.888 <sup>a</sup> (173.45-252.22)
EAA $t_{0-8}$ (mg/hour/L)	4699.791±260.754 <sup>a</sup> (4337.13-5133.22)	3566.447±454.691 <sup>b</sup> (2810.66-4122.43)	4120.218±506.797 <sup>c</sup> (3010.49-4473.37)	4113.364±180.547 <sup>c</sup> (3850.85-4375.40)
F (%)	-	0.759±0.096 <sup>a</sup> (0.60-0.88)	0.877±0.107 <sup>b</sup> (0.64-0.95)	0.875±0.038 <sup>b</sup> (0.82-0.92)

a, b, c, d. The difference between the groups with different letters in the same line is significant for SQ ( $p < 0.05$ );  $\alpha$ , speed constant of plasma drug; concentration dispersion period;  $\beta$ , curve of plasma drug concentration-time curve ejaculation period;  $t_{1/2\alpha}$ , semi-lifetime of absorption in ingestion canal in case of oral administration;  $t_{1/2\beta}$ , semi lifetime of a-period;  $t_{1/2\beta}$ , semi-lifetime of  $\beta$ -period; ART, the duration elapsed for ejaculation 63.2% of the drug from the body (average rest time);  $T_{max}$ , the duration elapsed for plasma drug concentration to the max value;  $Y_{max}$ , max drug concentration in plasma; EAA  $t_{0-8}$ , the area under the curve of plasma drug concentration and time; F, bioavailability

Table 2: Bioequivalence of B drug (test) to A drug (reference) (no logarithmic transformation and with logarithmic transformation) for SQ

	EAA	Log EAA	$T_{max}$	Log $t_{max}$	$Y_{max}$	Log $Y_{max}$
Test drug	4113.364±180.547	3.614	6.810±1.585	0.833	214.201±32.888	2.330
Reference drug	4120.218±506.797	3.614	5.870±1.559	0.768	226.301±19.569	2.354
Proportion	0.99	1	1.16	1.08	0.94	0.99
Bioequivalence acceptable limits	0.80-1.25	0.80-1.25	0.80-1.25	0.80-1.25	0.80-1.25	0.80-1.25

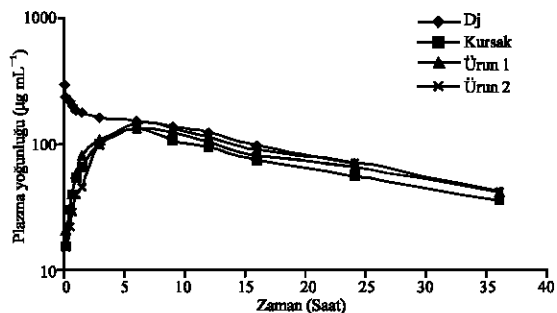


Fig. 1: Semi-logarithmic plasma concentration-time curve in cases of intravenous (Group I) and intracrop (Group II, III and IV) administration of SQ

## DISCUSSION

Recycling tests for SQ were done primarily within the scope of the study. As a result of these analyses, the recycling rate of the method was found to be 90.4%. This rate was similar to the rates found by Altıntaş (2006) and Elmas *et al.* (2000) (93, 98, 92 and 92%, respectively). However, it was found to differ from the results of the studies conducted by Jaouen *et al.* (1983) and Chakwenya *et al.* (2002) (104 and 68%, respectively). This

stems from differences in the method used. Sensitivity changes, on the other hand, are dependent on the method of analysis.

Materials were divided into 4 groups in the study. The first group was administered SQ standard solution through DI method. Group II was administered sulphaquinoxalin standard solution via the intracrop method and was used for determining the pharmacokinetic profile of these 2 drugs. Group III was administered reference drug while Group IV was administered the test drug. When the reference drug was compared to the test drug, the averages for EAA,  $Y_{max}$  ve  $t_{max}$  values were found EAA: 4120.218±506.797;  $Y_{max}$ : 226.301±19.569;  $t_{max}$ : 5.870±1.59 for the reference drug whereas they were found EAA: 4113.364±180.547;  $Y_{max}$ : 214.201±32.888;  $t_{max}$ : 6.810±1.85 for the test drug. These values were determined to be within acceptable limits (80-125%). After logarithmic transformation was applied on comparative pharmacokinetic parameters, the values were found EAA: 3.614;  $Y_{max}$ : 2.354;  $t_{max}$ : 0.768 for the reference drug while they were found EAA: 3.614;  $Y_{max}$ : 2.330;  $t_{max}$ : 0.833 for the test drug. These values were within acceptable limits (80-125%), indicating that, for therapeutic purposes, the 2 drug formulations could be regarded as equivalent.

In the study conducted by Altıntaş (2006), 2 products based on sulphonamid-TMP were compared. Evaluations were made separately for each drug substance. The measurements in this study conducted on winged animals were carried out via HPLC. As a result of the study, both drugs were evaluated on the condition that they were within 0.84-0.87 interval in terms of EAA and were regarded bioequivalent.

In addition, in the studies conducted by Murrieta *et al.* (1990) and Pokrajac *et al.* (1998) based on human drugs, sulphonamid-trimetophrim combinations were compared and found to be bioequivalent.

In the study conducted by the FDA (1993), 2 different preparations including sulphonamide-TMP were determined as bioequivalent in horses.

The bioavailability rate for Group II was 75.9%, for Group III it was 87.7% and for Group IV it was 87.5%. The bioavailability values of Group III and Group IV which were administered drugs were found to be higher than the values found by El-Sayed *et al.* (1995), Reddy *et al.* (1998), Lashev (1994) and Altıntaş (2006) (72.65, 60.6, 81 and 51.26%, respectively), while they were found lower than the value found by Şahindokuyucu (2003) (93.57%). These kinds of differences are assumed to stem from analytical methods as well as the additives and the combination varieties in the drugs.

In addition to bioequivalence, some pharmacokinetic parameters for SQ were calculated within the scope of the study. Accordingly, ART for Group I, Group II, Group III and Group IV were found to be  $24.085 \pm 0.917$ ;  $28.410 \pm 1.347$ ;  $29.958 \pm 1.934$  and  $28.437 \pm 0.979$ , respectively. In the study conducted by Altıntaş (2006) ART was calculated (7.90) in DI administration for SMZ (15.95) and in intracrop administration (12.84 and 10.52) for 2 different preparations of SMZ. The values in question were found to be lower than the ones in the study. This condition is thought to stem from the different analytical methods used and the fact that the sulphonamid type was different. Besides, the fact that ART was low in the group with DI administration among 4 groups can be explained by the short time elapsed in the organism of the drug with DI administration.

EAA was found ( $4699.791 \pm 260.754$ ) as a result of DI administration. This value was found to be higher than the value found for DI administration ( $389.43 \pm 20.6$ ) in the study conducted by Altıntaş (2006). In addition, when compared to the values found in the studies conducted by El Sayed *et al.* (1995), Reddy *et al.* (1998) and Queral and Castells (1985) were found to be higher ( $4078.8 \pm 143.21$ ;  $1429.13$  and  $31.220$ , respectively) while they were found lower than the value found by Şahindokuyucu (2003) ( $5090.2 \pm 194.4$ ).

EAA for Group II, Group III and Group IV was found to be  $3566.447 \pm 454.691$ ;  $4120.218 \pm 506.797$  and  $4113.364 \pm 180.547$ , respectively in the intracrop administration. These values were found to be higher than 2 preparations including SMZ ( $307.96 \pm 16.8$  and  $259.58 \pm 25.9$ ) and for intracrop administration ( $199.62 \pm 6.9$ ). When they were compared to the results of previous studies, they were found to be higher than the values found by El-sayed *et al.* (1995), Reddy *et al.* (1998) and Queral and Castells (1985) ( $2941.93 \pm 69.58$ ;  $1550.53$  and  $29.357$ , respectively) while they were found to be lower than the values found by Şahindokuyucu (2003) ( $4763.4 \pm 561.01$  and  $4260.0 \pm 115.5$ ).

When taken into consideration in terms of  $Y_{max}$  and  $t_{max}$  within pharmacokinetic parameters, these values in Group II, III and IV for  $Y_{max}$  were found to be  $196.070 \pm 33.712$ ;  $226.301 \pm 19.596$  and  $14.201 \pm 32.888$ , respectively while for  $t_{max}$  they were found  $6.128 \pm 0.623$ ;  $5.870 \pm 1.559$  and  $6.810 \pm 1.585$ , respectively. These values were determined to be higher than the results of the study conducted by Altıntaş (2006) (for 2 preparations containing SMZ; 21.24; 20.39 and 17.80, respectively). However, these results were found to be higher than those reported in the studies of Reddy *et al.* (1998) and Queral and Castells (1985) ( $146.31$  and  $54.50$  for  $Y_{max}$  and  $3.44$  and  $1.50$  for  $t_{max}$ ). The results of the present study were consistent with the value found by Altıntaş (2006) (6 h) in terms of  $t_{max}$  values.

The data obtained as a result of this study suggests that these 2 drugs were equivalent. Therefore, it was concluded that these drugs were interchangeable and might be substituted for one another.

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