

Fed and Fasting Bioequivalence Study for Two Formulations of Bosentan 125 Mg Tablets in Healthy Colombian People

Vargas M^{1*}, Bustamante C² and Villarraga EA³

¹Pharmacology Unit Universidad De La Sabana, Bogotá-Colombia

²Universidad La Sabana, Bogotá - Colombia

³Pharmacology Unit Universidad De La Sabana, Bogotá-Colombia

Abstract

This is a pharmacokinetic study of two formulations containing Bosentan 125 mg, in order to compare the bioavailability between the Test product (Bosentan produced by Tecnoquímicas S.A. laboratory, Colombia) and the Reference product (Tracleer[®] produced by Actelion Pharmaceuticals) in fasting and fed conditions, in order to state the bioequivalence between them. With this purpose, an open label, four periods, two randomized sequences, crossover, with single pre- and fed 125 mg dose study was performed in 30 healthy volunteers, with an 8-day washout period between each period and a collection of 14 plasma samples between 0 and 24 hours. Identification and evaluation of Bosentan in plasma was carried out by ultra-high-performance liquid chromatography-tandem mass spectrometry UHPLC/MS/MS as analytical method.

Based on the European and FDA bioequivalence research guidelines, the Confidence Interval (CI) falls within the allowed ranges for the Bioequivalence and Interchangeability Statement of the Tecnoquímicas S.A. product with the Reference product.

Both formulations had similar pharmacokinetic parameters in each studied condition, fed and fasted. Moreover, an increase in the amount of active pharmaceutical ingredient is evident in fed conditions.

Keywords: Bioequivalence; Bosentan; Pulmonary hypertension; Endothelin; Pharmacokinetics

Introduction

Bosentan is a dual endothelin receptor antagonist (ERA) with affinity for endothelin receptors A and B (ETA and ETB), diminishing pulmonary and systemic vascular resistance, and leading to an increase in cardiac output without increasing the heart rate [1-3].

Bosentan is a non-selective inhibitor of endothelin receptors A and B which, in short term studies, produced an improvement of the functional capacity and the distance travelled in the 6 minutes' walk test (6MWT) when compared to placebo [4,5]. Its clinical benefits have been demonstrated in specific groups such as Idiopathic Pulmonary Arterial Hypertension (PAH) [6], Connective Tissue Disease [7], Chronic Thromboembolic Pulmonary Hypertension (CTEPH) [8], and Congenital Heart Disease [9]. Among its virtues, in common with sildenafil, the convenience of the oral administration and the favorable adverse effects profile can be found. Henceforth, it is used as a first-line drug in patients with moderately symptomatic PAH, classically Functional Class III, to which Class II was subsequently added [10]. The objective of this study was to establish the Bioequivalence of two formulations of Bosentan 125 mg tablets by comparing the bioavailability after a single dose of the Test product produced by Tecnoquímicas S.A. (Colombia) and the Reference product, Tracleer[®], produced by Actelion Pharmaceuticals in fasting and fed conditions.

Materials and Methods

Study formulations

Test drug: Bosentan 125 mg tablet, manufactured and distributed in Colombia by Laboratorios Tecnoquímicas S.A. Lot: EX4E01.

Reference drug: Tracleer[®] Bosentan 125 mg tablet, manufactured and distributed by Actelion Pharmaceuticals & Biotoscana. Lot: EP100P0104.

Physicochemical properties such as active substance assay and dose-unit uniformity were evaluated for both the Test and Reference products, and they are summarized in Table 1 in order to state the Pharmaceutical Equivalence of these medicinal products before the conduction of the *in vivo* study.

Subjects

For the fasting and fed studies, 30 and 29 healthy volunteers were enrolled and completed the study, respectively. Volunteers were male, healthy, non-smokers, with an average age of 28 years old (18-46 years), average weight of 70 kg (59-84 kg), average height of 174 cm (160-184 cm), and an average Body Mass Index (BMI) of 23.1 kg/m² (19.1-25.9 kg/m²) (Table 2).

All volunteers were assessed with a medical examination and laboratory tests before the clinical phase to confirm their health status. Alcoholism history, preexistent diseases compromising liver or kidney function, blood dyscrasias or proteinuria were considered as exclusion criteria.

Medical examinations and clinical laboratory tests

The following tests were performed: complete blood-count, total and direct bilirubin, creatinine, glycaemia, total protein, complete

***Corresponding author:** Vargas M, Dentist, Pharmacologist, Scientific Director, Pharmacology Unit Universidad De La Sabana, Bogotá-Colombia, Tel: 861 5555; E-mail: mauricio.vargas@unisabana.edu.co

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Physicochemical characteristic	Test Product	Reference Product
Appearance	Capsular cores, with a 125 inscription in one side, grooved at the other, free from defects and strange particles.	Pale pink oblong tablet, 125 written at one side, free from strange particles.
Identification	The retention time of the sample is similar to the standard. Complies	The retention time of the sample is similar to the standard. Complies
Dose uniformity by weight variation	L less or equal to 15. L= 2.57	L less or equal to 15. L= 11.14
Dissolution	No less than the 80% of the declared amount is dissolved within 60 minutes. Average: 95%	No less than the 80% of the declared amount is dissolved within 60 minutes. Average: 93%
Assay - Bosentan	90.0% - 110.0% (125 mg labeled) 127.2 mg Tab 101.8%	90.0% - 110.0% (125 mg labeled) 134.7 mg Tab 107.7%

Table 1: Test and Reference product physicochemical tests results.

**DISSOLUTION PROFILE
BOSENTAN 125 mg PRODUCT
LABORATORIO TECNOQUÍMICAS**

REFERENCE PRODUCT: TRACLEER® ACTELION

1- DIFFERENTIATION FACTOR (f1)

$$f1 = \left[\frac{\sum_{i=1}^n |Rt - Tt|}{\sum_{i=1}^n |Rt|} \right] \times 100$$

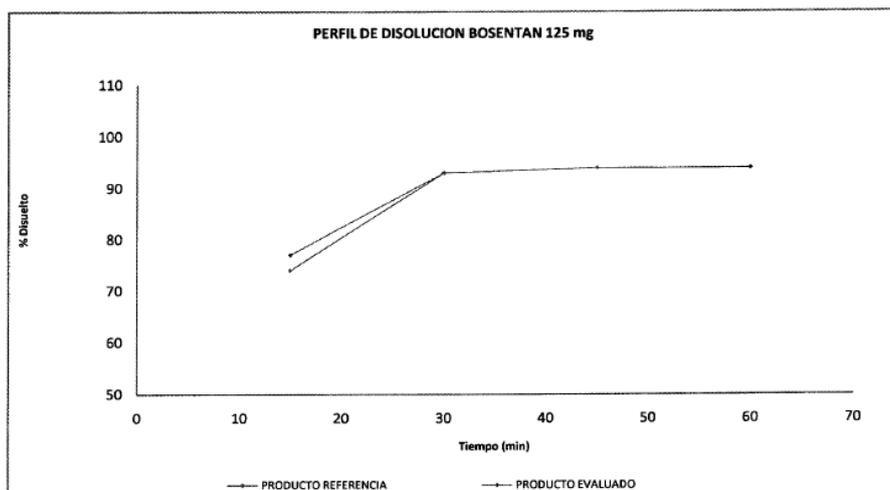
	Time	D% Reference	RSD	D% Product	RSD	f1	CONCEPT
1	15	74	3.7	77	5.6	4	COMPLIES
2	30	93	1.4	93	5.3	2	COMPLIES
3	45	94	1.6	94	4.3	1	COMPLIES
4	60	94	1.6	94	3.7	0.8	COMPLIES

1 DIFFERENTIATION FACTOR (f2)

$$f2 = 50 * \log \left\{ \left[1 + (1/n) \sum (R_i - T_i)^2 \right]^{-0.5} \times 100 \right\}$$

	Time	D% Reference	RSD	D% Product	RSD	f1	CONCEPT
1	15	74	3.7	77	5.6	75	COMPLIES
2	30	93	1.4	93	5.3	81	COMPLIES
3	45	94	1.6	94	4.3	85	COMPLIES
4	60	94	1.6	94	3.7	87	COMPLIES

BOSENTAN 125 mg DISSOLUTION PROFILE



Analista Responsable:

Reviso:

Luis Jose León C.

Ana Silvia Gamboa TQ

Luis Jose León Castillo Q.F.D.T

2014-10-03

Table 2: Results of the comparative dissolution profiles study for the Test and Reference formulations.

urinalysis, HIV ELISA test, antibodies against hepatitis B and C, and electrocardiogram.

Informed consent form

The Protocol and the Informed Consent Form were authorized by Universidad de la Sabana Clinical Research Ethics Committee (CEIC for its Spanish acronym) which is ruled by the legal and ethical guidelines of resolutions 008430 of 1993 and 002378 of 2008 of the Ministry of Social Protection (Colombia), the International Conference on Harmonization (ICH) for Good Clinical Practice of Institutions Conducting Investigation in Human Subjects and by the World Medical Assembly principles published in the Declaration of Helsinki [11].

The Informed Consent contains all the relevant points in compliance with the aforementioned requirements, and after a discussion the volunteers that decided to participate in the study signed such document.

Study design

A randomized, open-label, four periods, two sequences, crossover design was used with an 8-days washout period. Three days before each period initiation, volunteers must have refrained from consuming medications, alcohol and any food or beverage containing methylxanthines. These restrictions were maintained during the entire sampling period.

Drug administration

For the fasting study, volunteers underwent a 10 hours fasting period prior to the administration of a 125 mg single dose of Bosentan with 200 mL of water [12], and two hours later each volunteer was given a standardized food.

For the fed study, volunteers received a standardized breakfast and 30 minutes later the drug was administered in a single dose of 125 mg of Bosentan with 200 mL of water.

During hospital stay, they received three full meals (breakfast, lunch and dinner) and two snacks (one in the morning and one in the afternoon), all of them duly standardized by the Nutrition Department of the University Hospital of Universidad de La Sabana.

Using Vacutainer[®], a blood sample from each volunteer was obtained by venipuncture in the superior limb immediately prior to drug administration. Such sample was called 'zero time point sample'. In total, 14 blood samples were obtained from each volunteer at the following time points: 0, 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 12 and 24 hours. Samples were labeled for identification and centrifuged at 3000 rpm for 30 minutes. Plasma was transferred to a previously labeled tube and frozen at -20°C for subsequent analysis. After an 8-day washout period, administration was repeated completing the four study periods for fasting and fed studies.

Validation of analytical method

The validation was performed in compliance with the validation procedure of bioanalytical methodology established by QUASPAR M&F S.A. (PL-021) which meets the FDA suggestions to demonstrate a suitable specificity, linearity, precision, and accuracy [13]. The bioanalytical method employed for Bosentan quantification in plasma was ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) in electrospray ionization (ESI) mode [14-16].

Acetonitrile was employed as proteins precipitating agent. Analyte separation was achieved with a Zorbax Eclipse Plus C18 RHHT 2.1 x 5 mm, 1.8 µm Column, at 253 bar and a temperature of 40°C. Isocratic elution was performed with a mobile phase comprised by Formic Acid 0.1%: Acetonitrile 25:75, at a constant flow rate of 0.158 mL/min. Total run time was 2.5 min. Bosentan was detected with an ESI+ ionization mode, and monitoring the 552 ion. UHPLC injection volume was 2.0 µL.

Pharmacokinetic analysis

The pharmacokinetic analysis was performed using WinNonlin 5.3 (Pharsight Corporation, Cary USA) software, by means of a non-compartmental analysis. The peak concentration (C_{max}) and the time to peak concentration (T_{max}) were directly obtained from results of plasma concentrations, as currently recommended by the Food and Drug Administration (FDA) [17] and the European Medicines Agency (EMA) for drug assessments [18]. Total Area Under the Curve (AUC) was calculated by the sum of partial AUCs: a) AUC_{0-t}, between zero time point and the last time point with detectable concentrations, calculated by the trapezoidal rule and assuring the calculation of at least 80% of the AUC with the last sample, b) AUC_{t-∞}, calculated as the C/K ratio, where C is the last detectable concentration and K the slope obtained by linear regression from the points corresponding to the drug elimination phase through a linear regression of the natural logarithm of concentrations [17,18]. Bioavailability-adjusted elimination constant (K_e), half-life (t_{1/2}), clearance (Cl) and half mean residence time (MRT) were calculated after performing the non-compartmental analysis. The results of the pharmacokinetic variables are summarized in Table 3, including C_{max}, AUC_{0-t}, AUC_{0-∞}, T_{max} values and the elimination rate constant (K_e) of each one of the studied formulations.

Statistical analysis

An analysis of variance (ANOVA) was used to determine possible effects for each variation factor by sequence, period or subject. For this, F-test with a statistical significance level of 5% (α=0.05) was used. Statistical comparison of transformed pharmacokinetic parameters of both formulations was performed using the statistical software WinNonlin version 5.3. Bioequivalence criteria of FDA and EMA for AUC and C_{max} ratio between 80-125 after log-transformation with a 90% CI, were taken into account.

Adverse events report

The adverse events presented can be divided according to fasting or fed condition as follows: Fasting study: 6.7% blush, 5.0% headache, 3.3% epistaxis, and 1.7% sleepiness and chest pain on inspiration, all of mild severity. Fed study: 6.9% headache, 5.2% blush, and 1.7% sleepiness, all of mild severity. The events were registered and reported in compliance with INVIMA (Colombian regulatory authority) [19]. Since the sample size does not have enough statistical power, cases are informed as received from the research unit only and without any statistical estimation.

Results

Analytical results of active ingredient content, dose-unit uniformity and dissolution tests met the required specifications for the Pharmaceutical Equivalence statement, as shown in Table 1. The comparative dissolution profiles showed a differentiation factor (f₁) of 4.8, that falls within the compliance range of 0-15 and a similitude factor (f₂) of 82 which also falls within the compliance range of 50-100 for this variable (Table 2).

The analytical method showed to be specific, since no interferences arising from the matrix components were found in the identification and quantification of Bosentan. The precision was proven with variation coefficients less than 20% for low levels (25 ng/mL), and less than 15% for the medium (100 ng/mL) and high levels (500 ng/mL). The accuracy was determined by comparing the ratio between the areas of the samples against 5 calibration curves of the system obtaining deviations of less than 20% for the lowest concentration of the curves, and less than 15% for the rest of the concentrations, showing compliance with the specification. The quantification limit was 25 ng/mL and the detection limit was 0.1 ng/mL. The sample showed to be stable during the processing and thawing-freezing cycles.

The study involved the participation of 29 healthy Colombian volunteers who completed the four periods of the fasting and fed studies and were included in the pharmacokinetic and statistical analysis. Demographic data are summarized in Table 3.

Table 4 shows the averages of the pharmacokinetic parameters obtained from all volunteers (average \pm SD) for the fasting study, and Table 5 shows the same data for the fed study. Table 6 shows 90% Confidence Intervals of the log-transformed pharmacokinetic parameters for the fasting study, and Table 7 shows the same information for the fed study.

Demographic Variable	Obtained mean (n=29)
Age (years)	28 \pm 7.3
Height (cm)	174 \pm 5.4
Weight (kg)	70 \pm 6.6
BMI (kg/m ²)	23 \pm 2.3

Table 3: Demographics of Volunteers Included in the Pharmacokinetic and Statistical Analysis.

Parameter (unit)	Test Product	Reference Product
C_{max} (ng/mL)	1937.1 \pm 1068.1	2088.9 \pm 1344.6
AUC_{0-t} (ng.h/mL)	9407.8 \pm 3433.8	9300.1 \pm 4135.1
AUC_{0-∞} (ng.h/mL)	9892.0 \pm 3574.1	9654.8 \pm 4112.1
T_{max} (h)	3.2 \pm 1.5	2.7 \pm 0.9
Half-life time	3.7 \pm 1.5	3.7 \pm 1.5
Ke (1/h)	0.2 \pm 0.1	0.2 \pm 0.1

Table 4: Pharmacokinetic Parameters for Test Product Bosentan (Tecnocóquimicas S.A.) and Reference Product (Tracleer®) followed by a 125 mg Single Oral Fasting Dose

Parameter (unit)	Test Product	Reference Product
C_{max} (ng/mL)	2737.9 \pm 1185.7	3126.5 \pm 1422.9
AUC_{0-t} (ng.h/mL)	14367.3 \pm 5650.3	15038.0 \pm 5866.1
AUC_{0-∞} (ng.h/mL)	14650.2 \pm 5734.5	15277.4 \pm 5853.8
T_{max} (h)	3.6 \pm 1.6	3.0 \pm 1.1
Half-life time	3.0 \pm 1.1	2.8 \pm 0.8
Ke (1/h)	0.3 \pm 0.1	0.3 \pm 0.1

Table 5: Pharmacokinetic Parameters for Test Product Bosentan (Tecnocóquimicas S.A.) and Reference Product (Tracleer®) followed by a 125 mg Single Oral Fed Dose

	Units	Ratio%	Standard CI 90% (Test/Reference)	
Ln (C_{max})	ng / mL	95.5	83.0	109.7
Ln(AUC_{last})	hr*ng/mL	103.2	93.6	113.8
Ln(AUC_{all})	hr*ng/mL	103.9	94.8	113.8

Table 6: 90% Confidence Intervals of the log-transformed pharmacokinetic parameters for the two studied Bosentan formulations after fasting administration to healthy volunteers.

	Units	Ratio%	Standard CI 90% (Test/Reference)	
Ln (C_{max})	ng / mL	89.1	81.0	98.0
Ln(AUC_{last})	hr*ng/mL	95.9	89.0	103.3
Ln(AUC_{all})	hr*ng/mL	96.0	89.3	103.2

Table 7: 90% Confidence Intervals of the log-transformed pharmacokinetic parameters for the two studied Bosentan formulations after fed administration to healthy volunteers.

Discussion

Drug price control represents a constant effort for the governments [20], thus the use of multisource products showing safety and a good risk/benefit profile through validated investigations and, accordingly, Bioequivalence Studies allow the inference of interchangeability between generic drugs and reference products without repeating unnecessary large scale clinical trials in patients [21].

The guidelines for the Conduction of Comparative Bioavailability Studies by World Health Organization (WHO) recommend to carry out in vivo testing in multisource products to assess one dose and an increase of the drug plasma concentration, condition fulfilled by the present fasting and fed studies [9]. These findings are consistent with other studies which assess the pharmacokinetics changes of Bosentan when administered without food [22,23].

The in vitro study in which both studied formulations were compared, allowed the statement of Pharmaceutical Equivalence and the evaluation of the quality attributes of the formulations. The crossover, four periods, two sequences, single fasting and fed dose study design in healthy volunteers reduces the variability and allows the evaluation of the effect of food presence in the drug absorption process, as well as the comparison of the formulations in such conditions. The analytical method used was selective, precise, accurate and robust.

In total, 29 out of the 30 initially enrolled volunteers completed the study and non-serious adverse events arose neither from any of the two formulations nor from the fasting or fed conditions. The washout period was higher than the recommended 7 elimination half-lives and guaranteed the absence of carryover effect between periods.

The objective of this study was to assess the Bioequivalence of two Bosentan 125 mg formulations. In Figure 1, average plasma concentration-time curves are presented, where similarity can be observed between both products for the fasting and the fed condition. However, comparing different conditions, an increase of absorption in fed condition for both formulations can be observed. On the other hand, the biopharmaceutical parameters calculated fall within the FDA [17] and EMA [18] required interval in both fasting and fed conditions (Tables 6 and 7). This allows us to state Bioequivalence, and thus interchangeability between the two studied formulations.

In the case of Bosentan, the incorporation of a Bioequivalent formulation for the pharmacological treatment of Pulmonary Arterial Hypertension contributes to the clinical aim and price control of such pathology.

In response to this evidence, this study, conducted between the Test product manufactured by Tecnocóquimicas S.A. and Tracleer® manufactured by Actelion Pharmaceuticals in Colombian population, demonstrates that it will be possible to interchange these two formulations [17-19,24].

Both studied formulations presented similar pharmacokinetic parameters in each of the two conditions, fasting and fed, however an

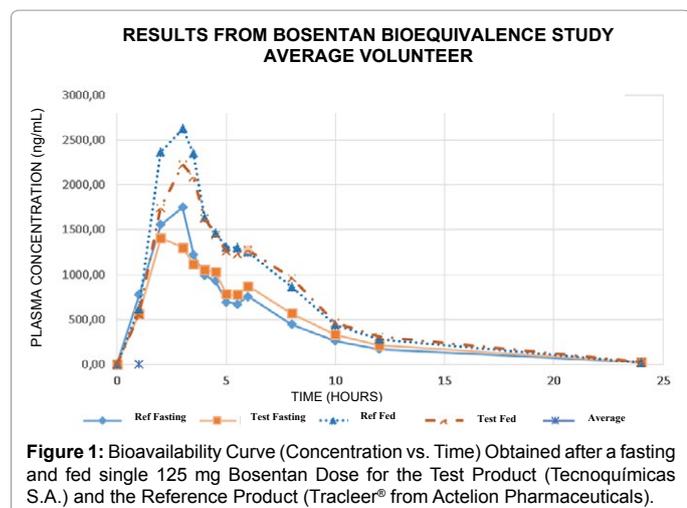


Figure 1: Bioavailability Curve (Concentration vs. Time) Obtained after a fasting and fed single 125 mg Bosentan Dose for the Test Product (Tecnoquímicas S.A.) and the Reference Product (Tracleer® from Actelion Pharmaceuticals).

Increase of the parameters in Fasting Vs. Fed condition				
FASTING	C_{max} Test	C_{max} Ref	AUC_{0-t} Test	AUC_{0-t} Ref
	1937.1	2088.9	9407.8	9300.1
FED	C_{max} Test	C_{max} Ref	AUC_{0-t} Test	AUC_{0-t} Ref
	2737.9	3126.5	14367.3	15038
Increase (%)	141%	150%	153%	162%

Table 8: Comparison among the pharmacokinetic parameters obtained with fasting and fed Bosentan 125 mg doses.

increase in the amount of active ingredient absorption can be evidenced in fed conditions, as shown in Table 8.

This finding is consistent with the study carried out by Dingemans et al. in 2002 about the influence of food intake in Bosentan pharmacokinetics [25], showing a percentage increase in C_{max} and AUC, but lesser than the values found in this study (22% and 10%, respectively). They also report a variation of approximately 100% with a greater dose (500 mg). Our study falls within the middle of the range of these results with an approximate variation of 50%.

Nonetheless, this variation should not be relevant from the clinical point of view since Bosentan possesses a broad therapeutic range, and a clear relationship between concentration and therapeutic effect has not been established yet [25].

Conclusions

In compliance with the EMA and FDA guidelines for Bioequivalence research, the Confidence Intervals for AUC and C_{max} ratios between the Bosentan formulation manufactured by Tecnoquímicas S.A. (Test product) and the formulation manufactured by Actelion Pharmaceuticals & Biotoscana (Tracleer®), (Reference product) fall within the allowed ranges for the Bioequivalence and Interchangeability Statement of the Tecnoquímicas S.A. product with the Reference product.

Both formulations had similar pharmacokinetic parameters in each studied condition, fed and fasted. Moreover, an increase in the amount of active pharmaceutical ingredient is evident in fed conditions.

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