

# Bioequivalence between two Prolonged Release Tablets of Desvenlafaxine Succinate in Healthy Subjects under Fasting and Fed Conditions

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## Abstract

Bioavailability in different formulations of desvenlafaxine 100 mg prolonged release tablets was compared in two bioequivalence studies, one under fasting conditions and the other after a standard breakfast. Both studies were single dose, randomized, open label, two-period crossover, with Brazilian male and female healthy subjects. Blood samples were taken during 48 h and plasmatic concentrations were determined using a validated UPLC-MS/MS method. Confidence intervals (CI90%) for the peak plasma concentration (C<sub>max</sub>) and area under the concentration-time curve (AUC<sub>0-t</sub>) were determined by calculating log-transformed data. In the fast study, the ratios and 90% CI for the geometric mean test/reference ratios were 101.45% (94.62-108.77%) for C<sub>max</sub> and 104.96% (100.24-109.89%) for AUC<sub>0-t</sub>. In the fed study, the ratios and 90% CI for the geometric mean was 110.24% (104.46-116.34%) for C<sub>max</sub> and 104.44% (99.97-109.11%) for AUC<sub>0-t</sub>. Under fasting and fed conditions, the test (desvenlafaxine succinate 100 mg prolonged release tablets, Zodiac Produtos Farmacêuticos S.A.) and reference (Pristiq™ 100 mg prolonged release tablets, Wyeth Indústria Farmacêutica Ltda) formulations were considered bioequivalent since the 90% CIs for the geometric mean test/reference ratios were within a predetermined range of 80% to 125% attending to FDA and ANVISA.

**Keywords:** Desvenlafaxine Succinate; Prolonged Release Tablets; Fasting and Fed Conditions; Bioequivalence; Chromatography

## Introduction

Major depressive disorder (MDD) is a functionally disabling condition characterized by depressed mood or loss of interest in the usual activities of daily life [1]. Desvenlafaxine (administered as desvenlafaxine succinate) is the major active metabolite of the serotonin-norepinephrine reuptake inhibitor venlafaxine after metabolism by CYP2D6; the free base of desvenlafaxine also is referred to as O-desmethylvenlafaxine. Desvenlafaxine, administered clinically as a succinate salt, is approved for the treatment of MDD [1,2,3].

The mean time to peak plasma concentration (t<sub>max</sub>) of desvenlafaxine after oral administration of extended release tablets is approximately 7.5 hours [4]. Desvenlafaxine absolute bioavailability is approximately 80% [3,5]. When desvenlafaxine was administered to healthy subjects under fasting conditions and after a high-fat meal, the C<sub>max</sub> increased approximately 16% in the fed state, and the AUCs were similar. The differences were not clinically significant [6]. Desvenlafaxine has linear and dose-proportional pharmacokinetics from 25 to 900 mg [3,6]. The protein binding of desvenlafaxine is low (30%), and its volume of distribution is approximately 3.4 L/kg. Desvenlafaxine metabolism primarily involves the non-CYP enzymes UGTs to form its glucuronide metabolite, which is also renally excreted. The UGTs are a family of enzymes involved in detoxification and inactivation of compounds, and their activity is generally spared in liver disease. The minor hepatic metabolic pathway involves CYP3A4 (<5%). Desvenlafaxine may have a low risk of variability in pharmacologic effect resulting from CYP2D6 polymorphisms or drug-drug interactions when coadministered with CYP2D6 substrates or inhibitors [1]. Renal excretion is an important route of desvenlafaxine elimination, with around 45% eliminated unchanged in the urine [1,6]. The elimination half-life (t<sub>1/2</sub>) of desvenlafaxine is about 11 hours [5]. The purpose of the studies in this paper was to compare, in healthy volunteers from both genders, the pharmacokinetic profiles of desvenlafaxine, aiming to assess the bioequivalence between two formulations: desvenlafaxine succinate 100 mg prolonged release film-coated tablet, manufactured by Zodiac Produtos Farmacêuticos S.A. (test drug) and Pristiq™ 100 mg prolonged release film-coated tablet, imported by Wyeth Indústria Farmacêutica Ltda (reference drug) under fasting and fed conditions.

Bioequivalence is one aspect of drug product quality that links *in vivo* performance of a reference product with a test formulation, demonstrating evidence of safety and efficacy. The essential pharmacokinetics of the active drug ingredient must be characterized. The pharmacokinetic parameters, which represent the rate ( $C_{max}$ ) and extent (AUC) of systemic absorption and elimination half-life ( $t_{1/2}$ ) should be established. When two formulations are bioequivalent, same rate and extent of absorption, the clinical efficacy and the safety profile of these drug products are assumed to be similar [7].

## Material and Methods

### Population

Twenty-eight (28) volunteers of both genders (14 female and 14 male subjects) aged between 18 and 50 years were screened for each study (fasting and fed conditions). All volunteers were considered as being eligible to participate in the studies and fulfilled all the inclusion and exclusion criteria defined in the protocols. All volunteers showed good health conditions or the absence of significant diseases after assessment of medical history, verification of vital signs, physical examination, electrocardiogram, and routine laboratory tests. All subjects enrolled in the studies showed negative tests for hepatitis B (HBsAg and Anti-HBc IgM), hepatitis C and HIV and urine HCG (pregnancy test only for female subjects). Both studies were conducted in compliance with guidelines and standards for researches involving human beings from Resolutions no. 466/12 and 251/97 by the National Health Council - Ministry of Health, Good Clinical Practices according to ICH, and the Document of the Americas and in compliance with the Declaration of Helsinki (adopted by the 18<sup>th</sup> WMA General Assembly in Helsinki/ Finland, 1964, and with the last amendment by the 64<sup>th</sup> WMA General Assembly in Fortaleza/ Brazil, 2013). The protocols (fasting and fed conditions) were submitted and approved before study start by the Research Ethics Committee of Faculdade de Jaguariúna in Jaguariúna, São Paulo, Brazil. After explaining the nature and purpose of the studies, all volunteers provided their written informed consent for participation.

### Study Treatments

In both studies (fasting and fed conditions), the test formulation was desvenlafaxine succinate monohydrate 100 mg prolonged release film-coated tablets (batch number 89134), manufactured by Zodiac Produtos Farmacêuticos S.A. Brazil, and the reference formulation was Pristiq<sup>TM</sup>, desvenlafaxine succinate monohydrate 100 mg prolonged release film-coated tablets (batch number 99768), manufactured by Pfizer Ireland Pharmaceuticals, Newbridge, Ireland.

### Study Design

The studies were conducted using an open-label, randomized, two-period, crossover, and balanced design, with a washout period of 14 days between administrations. In each of the study periods, the volunteers received a prolonged release film-coated tablet containing 100 mg of desvenlafaxine succinate from one of the two formulations mentioned above by orally, as a single dose with a 200-mL glass of water at room temperature. Just after study drug intake on both studies, ondansetron chloride 4 mg in 100 mL of saline solution 0.9% was administered via intravenous during 1 hour. This was a precaution measure to avoid adverse events caused by desvenlafaxine administration such as nausea and emesis. In the fasting condition study, the study drugs were administered after a minimum fasting of 8 hours. In the fed condition study, volunteers fasted for at least 8 hours and received the study drug 30 minutes after starting a standard breakfast (bread, butter, 2 slices of cheese, 2 slices of ham, 1 sweet wafers with strawberry stuffing, strawberry Petit Suisse yogurt and light artificial juice). In both studies, volunteers fasted for 4 hours after study drug administration. To maintain the standardization of treatment groups, the diet (food and drink) followed the same standard for all volunteers and in both periods. The intake of alcoholic beverages, food or beverages containing caffeine or xanthine (such as coffee, tea, chocolate and cola- or guarana-based soft drinks) was not permitted. In addition, the use of nicotine was prohibited from 48 hours before hospitalizations until the last blood draw, as well as any regular drugs (for at least 14 days) or occasional drugs (up to 7 days) before study start. Blood samples (7.5 mL) were collected in coated tubes, containing EDTA as anticoagulant. The collection schedule in both studies (fasting and fed conditions) included collections before (pre-dose) and 1:00; 2:00; 3:00; 4:00; 4:30; 5:00; 5:30; 6:00; 6:30; 7:00; 7:30; 8:00; 8:30; 9:00; 10:00; 12:00; 16:00; 24:00; 36:00 and 48:00 hours after the administration of each drug. A total of 21 blood samples were collected from each volunteer in each period. At fast and fed studies, right after collection, blood samples were centrifuged at 3,500 rpm for 10 minutes at approximately 4°C. Immediately after centrifugation, the plasma was separated and transferred to two previously labeled cryotubes. The tubes were stored in freezer at -20°C and were maintained at this temperature until analysis. Clinical, analytical, and statistical stages of the study were conducted by Centro Avançado de Estudos e Pesquisas Ltda. (CAEP), located in the city of Campinas, São Paulo, Brazil.

### Quantification of Desvenlafaxine in Human Plasma

Plasma concentrations of desvenlafaxine were determined using reversed-phase ultra-performance liquid chromatography with sequential mass spectrometry (RP-UPLC-MS/MS). The analytes were extracted from plasma using protein precipitation. Plasma sample (300 µL) was vortex mixed with 600 µL methanol 100%, after adding 50 µL of IS solution (desvenlafaxine-d6: 250 ng/mL). The mixture was centrifuged at 15000 rpm for 4 minutes, the supernatant was transferred to a vial containing 700 µL of 0.05% formic acid in water, and 5 µL was injected into the chromatographic system. To avoid inter-assay variations, all the samples from the same volunteer were quantified in the same analytical run. The detection parameter used was the mass-to-charge ratio ( $m/z$ ) between

precursor ions and product, and the quantification parameter was the ratio of areas under chromatogram peak identified in the retention time between analyte and internal standard. Desvenlafaxine concentrations in volunteer samples were calculated using interpolation in the calibration curve. The chromatographic analysis was conducted in an UPLC Acquity (Waters) with Waters column Acquity UPLC BEH Phenyl 2.1 x 50 mm, with a flow rate of 0.2 mL/min. The column was maintained at a temperature of 50°C, while the autoinjector was maintained at 10°C. The mobile phase used was 10mM ammonium acetate plus 0.1% formic acid and 100% methanol at a 70:30 ratio (v/v). The injection volume was 5 µL and the total run time set as 3 minutes. The mass spectrometry detection was conducted using electrospray ionization source in positive mode. The multiple reaction monitoring (MRM) method was used, and the transitions monitored were  $m/z$  264.28 > 58.09 and  $m/z$  270.28 > 58.08 for desvenlafaxine and desvenlafaxine-d6, respectively. The method was validated in compliance with ANVISA guidance for bioanalytical method validation, RDC Resolution no. 27, dated May 17, 2012 [8]. The method linearity ranged from 1 to 400 ng/mL. The validation parameters assessed were selectivity, linearity, intra- and inter-run precision, intra- and inter-run accuracy, matrix effect, residual effect, and stability of desvenlafaxine under different conditions.

## Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were obtained from the curves of plasma concentration vs. time for desvenlafaxine and statistically assessed for determination of bioequivalence, using the softwares Phoenix WinNonLin version 6.4 and Microsoft Excel version 2007. The area under the curve of plasma concentration vs. time was calculated using the trapezoidal method, from time zero to the last measurable concentration ( $AUC_{0-t}$ ). The area under the curve of plasma concentration vs. time was also calculated from time zero to infinity ( $AUC_{0-\infty}$ ), where  $AUC_{0-\infty} = AUC_{0-t} + Ct/Kel$ , with  $Ct$  being the last drug concentration experimentally defined and  $Kel$  being the terminal phase elimination rate constant. The peak of maximum plasma concentration ( $C_{max}$ ) of desvenlafaxine and the time to reach this peak ( $t_{max}$ ) were obtained directly with no data interpolation. The elimination half-life ( $t_{1/2}$ ) was defined using the equation  $t_{1/2} = \ln 2 / Kel$  [2]. For the assessment of bioequivalence between formulations, AUC and  $C_{max}$  parameters were used. A 90% Confidence Interval (CI) was generated for the difference in averages of transformed data from test and reference drugs. The antilog of obtained CI comprised the 90% CI for geometric mean ratio of primary parameters. The drugs are considered as bioequivalent if the extremities of CI generated for the geometric mean ratio for both primary parameters are higher than 80% and lower than 125%, as recommended by ANVISA (RDC 1170/12) and FDA [9,10].

## Results

In the fasting condition study, out of 28 participant volunteers, 23 completed the two study periods. In the fed condition study, out of 28 enrolled volunteers, 24 completed the study. The volunteers participating in the bioequivalence study under fasting conditions had mean age of 32 years, ranging from 18 to 50 years; mean weight of 69.8 kg (55.4 to 89.3 kg); mean height of 1.67 m (1.46 to 1.85 m) and mean BMI of 24.87 kg/m<sup>2</sup> (21.15 to 29.93 kg/m<sup>2</sup>). The volunteers participating in the fed bioequivalence study had mean age of 29 years, ranging from 18 to 49 years; mean weight of 69.0 kg (50 to 90 kg); mean height of 1.68 m (1.53-1.83 m) and mean BMI of 24.52 kg/m<sup>2</sup> (19.41 to 29.62 kg/m<sup>2</sup>). Desvenlafaxine was well tolerated at the administered dose in both studies. No serious adverse events were seen or reported, and no pregnancies were detected. The most common adverse event was headache, reported by 20% of the volunteers in the fasting condition study and by 30% of the volunteers in the fed condition study. The mean curves for plasma concentration vs. time obtained for test and reference drugs are shown in Figures 1 (fasting condition) and 2 (fed condition). The curves were shown to be overlapped, showing a similar pharmacokinetic profile between the drugs for both studies.

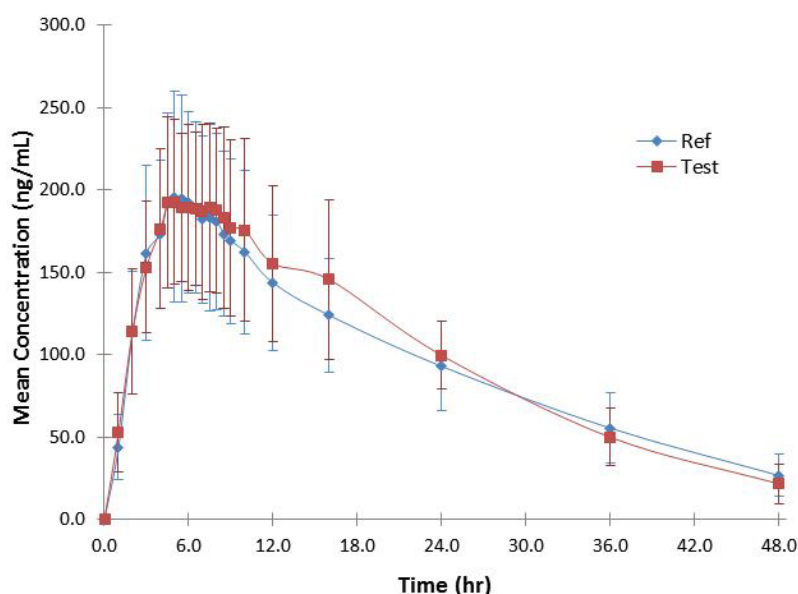


Figure 1: Mean curves of desvenlafaxine plasma concentration vs. time obtained for test and reference drugs under fasting conditions.

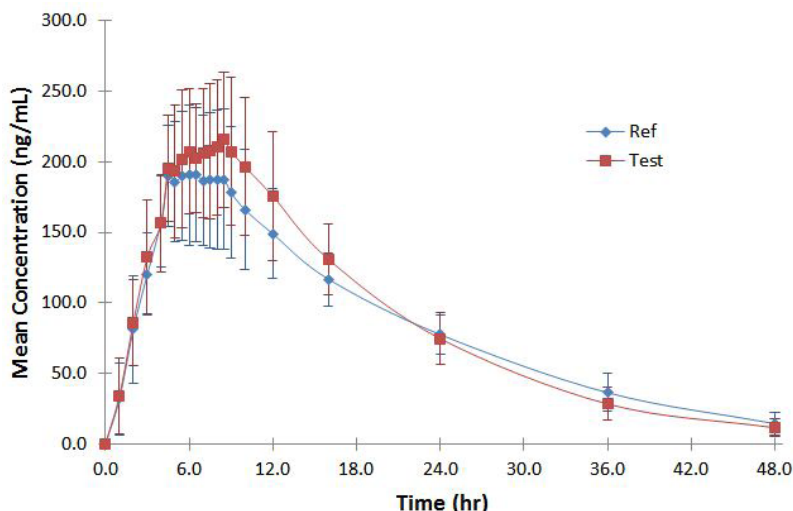


Figure 2: Mean curves of desvenlafaxine plasma concentration vs. time obtained for test and reference drugs under fed conditions.

The central location and dispersion measures for all pharmacokinetic parameters from formulations are shown in Tables 1 (fasting condition) and 2 (fed condition).

Parameter	Test Drug	Reference Drug
Cmax (ng/mL)	209.96 ± 11.67	209.35 ± 13.30
tmax (h)	6.59 ± 0.6	5.80 ± 0.36
AUC <sub>0-t</sub> (ng*h/mL)	4762.72 ± 216.23	4559.57 ± 231.58
AUC <sub>0-∞</sub> (ng*h/mL)	5195.24 ± 279.25	5290.12 ± 295.40
t½ (h)	11.54 ± 1.01	15.53 ± 1.11

Table 1: Pharmacokinetic parameters (mean ± standard deviation) of desvenlafaxine obtained after oral administration under fasting conditions.

Cmax: maximum plasma concentration; tmax: time to reach the maximum plasma concentration; AUC<sub>0-t</sub>: area under the curve of plasma concentration vs. time from time 0 to t; AUC<sub>0-∞</sub>: area under the curve of plasma concentration vs. Time from time 0 to infinity; t½: elimination half-life

Parameter	Test Drug	Reference Drug
Cmax (ng/mL)	234.55 ± 8.46	213.08 ± 8.10
tmax (h)	7.23 ± 0.43	6.17 ± 0.36
AUC <sub>0-t</sub> (ng*h/mL)	4205.35 ± 137.20	4014.35 ± 117.11
AUC <sub>0-∞</sub> (ng*h/mL)	4365.06 ± 147.59	4276.79 ± 135.68
t½ (h)	8.76 ± 0.40	10.50 ± 0.68

Table 2: Pharmacokinetic parameters (mean ± standard deviation) of desvenlafaxine obtained after oral administration under fed conditions.

Cmax: maximum plasma concentration; tmax: time to reach the maximum plasma concentration; AUC0-t: area under the curve of plasma concentration vs. time from time 0 to t; AUC0-∞: area under the curve of plasma concentration vs. Time from time 0 to infinity; t½: elimination half-life

Tables 3,4 show the test/reference geometric mean ratios for pharmacokinetic parameters Cmax, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> and the respective 90% CIs for the bioequivalence analysis of the studies. All 90% CIs were within the interval of 80% to 125%.

Parameters	Geometric mean ratio (%)	Confidence interval (90%)	Intra-subject coefficient of variation (%)	Test power (%)
Ln(Cmax)	101.45	94.62-108.77	13.68	99.95
Ln(AUC <sub>0-t</sub> )	104.96	100.24-109.89	9.00	100.00
Ln(AUC <sub>0-∞</sub> )	98.07	93.91-102.41	8.48	100.00

Table 3: Geometric mean ratio and confidence intervals (90%) of desvenlafaxine test and reference drugs for assessment of bioequivalence under fasting conditions

Parameters	Geometric mean ratio (%)	Confidence interval (90%)	Intra-subject coefficient of variation (%)	Test power (%)
Ln(C <sub>max</sub> )	110.24	104.46-116.34	10.86	99.99
Ln(AUC <sub>0-t</sub> )	104.44	99.97-109.11	8.81	100.00
Ln(AUC <sub>0-∞</sub> )	101.84	97.42-106.45	8.92	100.00

**Table 4:** Geometric mean ratio and confidence intervals (90%) of desvenlafaxine test and reference drugs for assessment of bioequivalence under fed conditions.

## Discussion

Drug quantification in blood, serum, and plasma for the conduction of pharmacokinetic or bioequivalence studies requires sensitive and selective bioanalytical methods that are able to quantify reduced drug concentrations in the range of ng/mL or pg/mL, with no significant interferences from biological matrices [11]. With the advantages of a short analysis cycle, strong separation capacity, high resolution and sensitivity, precision in quantification of molecular compounds and rapid identification, the methods using ultra-performance liquid chromatography (UPLC) have been widely used in studies of pharmaceutical compounds related to small organic molecules, proteins, peptides, alkaloids, and natural compounds. The mass spectrometry detector brings high selectivity and sensitivity to the method [12,13].

With the purpose of obtaining a highly sensitive and rapid method for quantification of desvenlafaxine in plasma, a method by UPLC-MS/MS was developed and validated in this project. In the presented method the limit of quantification was 1 ng/mL, which allowed for a sensitive and efficient analysis of desvenlafaxine plasma concentrations. Two drugs are considered bioequivalent if their rate and extent of absorption do not show statistically significant differences when administered at the same molar dose of the active ingredient, under the same experimental conditions [14]. In these studies, the relative bioavailability of two formulations of desvenlafaxine was assessed after administration under fasting and fed conditions. Desvenlafaxine pharmacokinetic results (C<sub>max</sub>, AUC, t<sub>max</sub> and t<sub>1/2</sub>) found in the studies (Tables 1 and 2) were very similar to those reported on the literature under fasting and fed conditions [2,3,6]. As shown in Tables 3 and 4, 90% CIs obtained for pharmacokinetic parameters defining bioequivalence (C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub>) of desvenlafaxine 100 mg were shown to be within the bioequivalence limits defined by ANVISA (80%-125%) in RE Resolution no. 1170, dated April 19, 2006 (9).

## Conclusion

In both bioequivalence studies, one with administration under fasting conditions and the other with administration under fed conditions (standard breakfast), based on pharmacokinetic and statistical results obtained, we conclude that the test drug (desvenlafaxine succinate 100 mg - Zodiac Produtos Farmacêuticos S.A.) and the reference drug (Pristiq™ 100 mg – Wyeth Indústria Farmacêutica Ltda) are bioequivalent. Thus, desvenlafaxine 100 mg prolonged release film-coated tablets may be considered as being interchangeable in medical practice, since they have the same efficacy and safety profile for the patients.

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