Bioequivalence Between Two Prolonged Release Tablets of Darifenacin in Healthy Subjects Under Fasting and Fed Conditions

Valery Aleksandravicius de Carvalho Accennato¹, Karla Cristina Lima da Silva Oliveira¹, Fernando Costa¹, Patricia Maróstica¹, Ricardo Horácio Pires¹, Maria Francesca Riccio², Gilberto Bernasconi², Ana Cláudia Noboli²

¹Zodiac Produtos Farmacêuticos S/A. Pindamonhangaba – São Paulo - Brazil
²CAEP - Centro Avançado de Estudos e Pesquisas. Campinas – São Paulo- Brazil

Corresponding Author: Valery de Carvalho
vale.carvalho@zodiac.com.br

ABSTRACT

Bioavailability in different formulations of darifenacin 15 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 males and females healthy subjects. Blood samples were taken during 72 h and plasmatic concentrations were determined using a validated UPLC-MS/MS method. Confidence intervals (CI90%) for the peak plasma concentration (Cmax) and area under the concentration-time curve (AUC0-t) were determined by calculating log-transformed data. In the fast study, the ratios and 90% CI for the geometric mean was 94.82% (86.08-104.46%) for Cmax and 92.49% (84.68-101.03%) for AUC0-t. In the fed study, the ratios and 90% CI for the geometric mean was 94.41% (85.96-103.70%) for Cmax and 94.37% (88.79-100.31%) for AUC0-t. Under fast and fed conditions, the test (darifenacin 15 mg prolonged release tablets, Zodiac Produtos Farmacêuticos S.A.) and reference (Enablex® 15 mg prolonged release tablets, Novartis Biociências S.A.) formulations were considered bioequivalent since the 90% CIs for the geometric mean test/reference ratios were within a predetermined range of 80% to 125% according to FDA and to ANVISA.

Keywords: darifenacin, prolonged release tablets, fasting and fed conditions, bioequivalence, chromatography

1. INTRODUCTION

Darifenacin hydrobromide, chemical name alpha-(3S)-1-[2-(2,3-dihydro-5-benzofuranyl) ethyl], is a strong and competitive muscarinic receptor antagonists with selective affinity to M3 cholinergic receptors. As indicated by its chemical name, darifenacin contains an asymmetric center, but it was developed as a single enantiomer, (S), which is the eutomer of the racemic mixture. Darifenacin is used in the treatment of overactive bladder syndrome, a benign and persistent pathology, characterized by the increased urination frequency, urination urgency, and urinary incontinence, particularly related to advanced age, mainly mediated by the activation of M3 cholinergic muscarinic receptors present in the urinary bladder (1,2,3,4). The main therapy for this syndrome is the use of anticholinergic agents, which play their role by blocking M3 muscarinic receptors located in urinary bladder smooth muscle (4).

After oral administration, darifenacin is rapidly and fully absorbed (close to 97%). Darifenacin absorption rate from extended release tablets is limited by the release rate from the matrix drug, resulting in a delayed absorption that occurs mainly in the colon (1). Its bioavailability is very low, approximately 15-19%, due to the extensive first-pass metabolism when administered by the oral route (1,3). In healthy volunteers, the peak of maximum plasma concentration (Cmax) of darifenacin occurs between 5.5 and 11.5 hours (tmax) after oral administration of extended release tablets (1).

The administration of darifenacin extended release tablets with a high-calorie diet increased in 22% the Cmax and decreased the tmax mean in 3.3 hours when compared to the administration under fasting conditions. However, no clinically significant increases were seen in the pharmacokinetic parameter of AUC when the drug was administered under both conditions (1).

Darifenacin is highly bound to plasma proteins (98%), mainly to alpha-1-acid glycoprotein. Due to its lipophilicity, it shows a high volume of distribution, between 165 to 276 L. Darifenacin undergoes extensive liver metabolism by cytochromes CYP2D6 and CYP3A4, with 3% of unchanged drug being excreted in the urine and feces. The elimination half-life (t½) of darifenacin is between 13 and 19 hours (1,3).

The purpose of the studies in this paper was to compare, in healthy volunteers from both genders, the pharmacokinetic profiles of darifenacin, aiming at assessing the bioequivalence between two formulations: darifenacin hydrobromide 15 mg extended release film-coated tablet, registered by Zodiac Produtos Farmacêuticos S.A. (test drug) and Enablex® 15 mg extended release film-coated tablet, imported by Novartis Biociências S.A. (reference drug) under fasting and fed conditions.

2. MATERIAL AND METHODS

Population
Seventy-six (76) volunteers of both genders (38 female 38 male subjects) aged between 18 and 50 years were screened for each study (fasting and fed conditions). There were no restrictions for ethnic group. 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).

The study was 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 18th WMA General Assembly in Helsinki/ Finland, 1964, and with the last amendment by the 64th 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 Hospital Irmãos Penteado (Irmandade de Misericórdia) in Campinas, 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 darifenacin hydrobromide 15 mg extended release film-coated tablets (batch number 87680), manufactured by Zodiac Produtos Farmacêuticos S.A. Brazil, and the reference formulation was Enablex®, darifenacin hydrobromide 15 mg extended release film-coated tablets (batch number 50220), manufactured by Novartis Pharma Stein AG Switzerland.

Study Design
The purpose of the studies was to compare the bioavailability of two formulations of darifenacin hydrobromide 15 mg extended release film-coated tablets, under fasting and fed conditions. The studies were conducted using an open-label, randomized, two-period, cross-sectional, and balanced design, with a washout period of 14 days between administrations. In each of the study periods, the volunteers received an extended release film-coated tablet containing 15 mg of darifenacin hydrobromide from one of the two formulations mentioned above (dispensed in vials protected from light) by oral route, as a single dose with a 200-mL glass of spring water at room temperature. In the fasting condition study, the
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. In both studies, volunteers fasted for 4 hours after drug administration. In order 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 irregular drugs (up to 7 days) before study start.

Blood samples (7.5 mL) were collected in coated tubes for light block, containing EDTA as anticoagulant. The collection schedule in the fasting condition study included collections before (pre-dose) and 1:00; 2:00; 4:00; 5:00; 6:00; 6:30; 7:00; 7:30; 8:00; 8:30; 9:00; 9:30; 10:00; 10:30; 11:00; 11:30; 12:00; 13:00; 14:00; 16:00; 24:00; 36:00; 48:00, and 72:00 hours after the administration of each drug. A total of 25 blood samples were collected from each volunteer in each period. The fed condition study schedule had collections before (pre-dose) and 1:00; 2:00; 4:00; 5:00; 6:00; 6:30; 7:00; 7:30; 8:00; 8:30; 9:00; 9:30; 10:00; 11:00; 12:00; 14:00; 16:00; 24:00; 36:00; 48:00, and 72:00 hours after the administration of each drug. A total of 22 blood samples were collected from each volunteer in each period.

In both 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 amber cryotubes. The tubes were stored in ultralow temperature freezer at -70° C and were maintained at this temperature until the 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 darifenacin in human plasma**

Plasma concentrations of darifenacin were determined using reversed-phase ultra-performance liquid chromatography with sequential mass spectrometry (RP-UPLC-MS/MS). The analytes were extracted from plasma using liquid-liquid extraction with methyl tert-butyl ether solvent. Deuterated darifenacin was used as the internal standard. In order to avoid inter-assay variations, all the samples from the same volunteer were assessed 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. Darifenacin concentrations in volunteer samples were calculated using interpolation in the calibration curve. The chromatographic analysis was conducted in a UPLC Acquity (Waters) with Waters column Acquity UPLC BEH C18 2.1 x 50 mm, with a flow rate of 0.3 mL/min. The column was maintained at a temperature of 30° C, while the autoinjector was maintained at 10° C. The mobile phase used was 0.1% ammonium hydroxide and 100% methanol at a 30:70 ratio (v/v). The injection volume was 5 μL and the total run time set as 3.5 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 427 > 147 and m/z 431 > 151 for darifenacin and darifenacin-d4, respectively.

The method was validated in compliance with ANVISA guidance for bioanalytical method validation, RDC Resolution no. 27, dated May 17, 2012 (5). The method linearity range was from 20 to 10,000 pg/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 darifenacin under different conditions.

**Pharmacokinetic and Statistical Analysis**

The pharmacokinetic parameters were obtained from the curves of plasma concentration vs. time for darifenacin and statistically assessed for determination of bioequivalence, using Phoenix WinNonLin version 6.4 and Microsoft Excel version 2007 softwares. The area under the curve of plasma concentration vs. time was calculated using the trapezoidal method, from time zero to the last measurable concentration (AUC0-t). The area under the curve of plasma concentration vs. time was also calculated from time zero to infinity (AUC0-∞), where AUC0-∞ = AUC0-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 (Cmax) of darifenacin and the time to reach this peak (tmax) were obtained directly with no...
data interpolation. The elimination half-life \( (t_{1/2}) \) was defined using the equation \( t_{1/2} = \ln(2) / K_e \).

For the assessment of bioequivalence between both formulations, AUC and Cmax parameters were used. No volunteers whose parameters were discrepant when compared to the others were withdrawn from the statistical analysis. 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 are higher than 80% and lower than 125%, as recommended by ANVISA and FDA (6,7).

3. RESULTS

In the fasting condition study, out of 76 participant volunteers, 60 completed the two study periods. In the fed condition study, out of 76 enrolled volunteers, 67 completed the study. The volunteers participating in the bioequivalence study under fasting conditions had mean age of 30.35 years, ranging from 18 to 50 years; mean weight of 70.5 kg (46 to 98 kg); mean height of 1.67 m (1.47 to 1.88) and mean BMI of 25.26 kg/m² (18.90 to 30.77) kg/m². The volunteers participating in the bioequivalence study after feeding had mean age of 32.38 years, ranging from 19 to 49 years; mean weight of 69.3 kg (49.7 to 104.4 kg); mean height of 1.68 m (1.48-1.89) and mean BMI of 24.49 kg/m² (18.87 to 29.86) kg/m².

Darifenacin was well tolerated at the administered dose in both studies. No adverse events were seen or reported, and no pregnancies were detected during the studies. The most common adverse event was headache, reported by 23.2% of the volunteers in the fasting condition study and by 22.3% 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 virtually overlapped, showing a similar pharmacokinetic profile between the drugs for both studies.

The mean curves for plasma concentration vs. time obtained for test and reference drugs under fasting conditions are shown in Figures 1 (fasting condition) and 2 (fed condition).

![Figure 1: Mean curves of darifenacin plasma concentration vs. time obtained for test and reference drugs under fasting conditions](image-url)
Figure 2: Mean curves of darifenacin plasma concentration vs. time obtained for test and reference drugs under fed conditions

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Drug</th>
<th>Reference Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (pg/mL)</td>
<td>2340.55 ± 1581.15</td>
<td>2493.85 ± 1933.53</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>9.66 ± 5.43</td>
<td>9.55 ± 5.50</td>
</tr>
<tr>
<td>ASC0-t (pg*h/mL)</td>
<td>53311.96 ± 37470.13</td>
<td>55426.85 ± 35830.14</td>
</tr>
<tr>
<td>ASC0-∞ (pg*h/mL)</td>
<td>58684.80 ± 36967.84</td>
<td>59089.14 ± 36616.21</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>10.84 ± 5.12</td>
<td>12.01 ± 6.10</td>
</tr>
</tbody>
</table>

Cmax: maximum plasma concentration; tmax: time to reach the maximum plasma concentration; ASC0-t: area under the curve of plasma concentration vs. time from time 0 to t; ASC0-∞: area under the curve of plasma concentration vs. Time from time 0 to infinity; t½: elimination half-life

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Drug</th>
<th>Reference Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (pg/mL)</td>
<td>3844.98 ± 3786.89</td>
<td>4331.82 ± 4548.79</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>6.72 ± 3.84</td>
<td>8.20 ± 5.26</td>
</tr>
<tr>
<td>ASC0-t (pg*h/mL)</td>
<td>61666.26 ± 58215.92</td>
<td>64147.77 ± 53749.19</td>
</tr>
<tr>
<td>ASC0-∞ (pg*h/mL)</td>
<td>63694.42 ± 59414.86</td>
<td>66483.36 ± 56166.33</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>9.66 ± 7.70</td>
<td>9.31 ± 5.15</td>
</tr>
</tbody>
</table>

Cmax: maximum plasma concentration; tmax: time to reach the maximum plasma concentration; ASC0-t: area under the curve of plasma concentration vs. time from time 0 to t; ASC0-∞: area under the curve of plasma concentration vs. Time from time 0 to infinity; t½: elimination half-life

Tables 3 and 4 show the test/reference geometric mean ratios for pharmacokinetic parameters Cmax, ASC0-t, and ASC0-∞ and the respective 90% CIs for the bioequivalence analysis of the studies. All 90% CIs were within the interval of 80% to 125%.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Geometric mean ratio (%)</th>
<th>Confidence interval (90%)</th>
<th>Intra-subject coefficient of variation (%)</th>
<th>Test power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln(Cmax)</td>
<td>94.82</td>
<td>86.08-104.46</td>
<td>32.45</td>
<td>98.34</td>
</tr>
<tr>
<td>Ln(ASC0-t)</td>
<td>92.49</td>
<td>84.68-101.03</td>
<td>29.47</td>
<td>99.33</td>
</tr>
<tr>
<td>Ln(ASC0-∞)</td>
<td>95.41</td>
<td>87.68-103.83</td>
<td>26.51</td>
<td>99.59</td>
</tr>
</tbody>
</table>

Table 4: Geometric mean ratio and confidence intervals (90%) of darifenacin test and reference drugs for assessment of bioequivalence after administration under fed conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Geometric mean ratio (%)</th>
<th>Confidence interval (90%)</th>
<th>Intra-subject coefficient of variation (%)</th>
<th>Test power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln(Cmax)</td>
<td>94.41</td>
<td>85.96-103.70</td>
<td>33.41</td>
<td>98.77</td>
</tr>
<tr>
<td>Ln(ASC0-t)</td>
<td>94.37</td>
<td>88.79-100.31</td>
<td>21.40</td>
<td>99.99</td>
</tr>
<tr>
<td>Ln(ASC0-∞)</td>
<td>95.26</td>
<td>89.56-101.31</td>
<td>20.91</td>
<td>99.99</td>
</tr>
</tbody>
</table>

4. 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 (8). 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 (9,10).

With the purpose of obtaining a highly sensitive and rapid method for quantification of darifenacin in plasma, a method by UPLC-MS/MS was developed and validated in this project. In the presented method the limit of quantification was 20 pg/mL, which allowed for a sensitive and efficient analysis of darifenacin plasma concentrations.

Two drugs are considered as being bioequivalent if their absorption extensions and rates do not show statistically significant differences when administered at the same molar dose of the active ingredient, under the same experimental conditions (11). In the studies of this paper, the relative bioavailability of two formulations of darifenacin was assessed after administration under fasting and fed conditions. The pharmacokinetic results (Cmax, AUC, tmax and t½) found in the studies for darifenacin (Tables 1 and 2) were very similar to those reported by Skerjanec, 2006 (1) under fasting and fed conditions. As shown in Tables 3 and 4, 90% CIs obtained for pharmacokinetic parameters defining bioequivalence (Cmax, ASC0-t, and ASC0-∞) of formulations of darifenacin 15 mg were shown to be within the bioequivalence limits defined by ANVISA (80% - 125%) in RE Resolution no. 1170, dated April 19, 2006 (6).

5. CONCLUSION

In both bioequivalence studies conducted, 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 (darifenacin hydrobromide 15 mg - Zodiac Produtos Farmacêuticos S.A.) and the reference drug (Enablex® 15 mg – Novartis Biociências S.A.) are bioequivalent. Thus, darifenacin 15 mg extended 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.
REFERENCES