Bioequivalence and Pharmacokinetics of Two Formulations of Amlodipine Tablets in Healthy Subjects

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Abstract

The bioequivalence of a single dose tablet containing 5 mg amlodipine as a test product in comparison to Norvasc® 5 mg tablet (Pfizer USA) as the reference product was studied. Both products were administered to twenty eight healthy male adult subjects applying a fasting, single-dose, two-treatment, two-period, two-sequence, randomized crossover design with two weeks washout period between dosing. Twenty blood samples were withdrawn from each subject over 144 hours period. Amlodipine concentrations were determined in plasma by a validated HPLC-MS/MS method. From the plasma concentration-time data of each individual, the pharmacokinetic parameters; $C_{\text{max}}$, $T_{\text{max}}$, AUC$_{0-t}$, AUC$_{0-\infty}$, $C_{\text{max}}$/AUC$_{0-\infty}$, $\lambda_z$, T$_{0.5}$, MRT, Cl/F and Vd/F; were calculated applying non-compartmental analysis. The average values of the above parameters for the test formula were 1.99 ng/ml, 8.3 hours, 82.87 ng.hr/ml, 95.23 ng.hr/ml, 0.0219 hr$^{-1}$, 0.018 hr$^{-1}$, 38.5 hr, 56.2 hr, 60.9 l/hr and 3483 liters, respectively. The average values of these parameter for the reference formula were 1.92 ng/ml, 7.9 hours, 76.3 ng.hr/ml, 89.31 ng.hr/ml, 0.0225 hr$^{-1}$, 0.019 hr$^{-1}$, 36.7 hr, 59.9 hr, 69.5 l/hr, and 3983.4 liters, respectively. The pharmacokinetic parameters mentioned above were statistically analyzed by ANOVA test. Ln-transformed values of the pharmacokinetic parameters used for bioequivalence testing; $C_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$ were also statistically analyzed by ANOVA, 90% Confidence Interval (CI) and Schuirmann’s two one-sided t-tests. For the $T_{\text{max}}$, parametric and nonparametric tests were applied. Based on FDA criteria on bioequivalence, the results of the above statistical tests demonstrated bioequivalence of the two products.

Keywords: Amlodipine, Pharmacokinetic, Bioequivalence, HPLC/MS/MS.

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دراسة التكافؤ الحيوي وحركية الدواء لمستحضرين من أقراص الاملودبين على متطوعين اصحاء

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الخلاصة

تم دراسة التكافؤ الحيوي وحركية الدواء لمستحضر جينس على شكل حبوب يحتوي على خمسة مليغرامات من دواء الاملودبين بالمقارنة مع المستحضر المرجعي نورفاسك على شكل حبوب يحتوي أيضا على خمسة مليغرامات من دواء الاملودبين و المنتج من شركة فيزر الأمريكية. تم اعطاء كل المتطوعين لثمانية وعشرون شربة من المنتجين على التوالي وتم استخدام طريقة HPLC/MS/MS كطريقة في تحليل المكونات الدوائية. وتم حساب عوامل حركية الدواء في الجسم وهي: $C_{\text{max}}$, $T_{\text{max}}$, AUC$_{0-t}$, AUC$_{0-\infty}$, $C_{\text{max}}$/AUC$_{0-\infty}$, $\lambda_z$, T$_{0.5}$, MRT, Cl/F, Vd/F وكان النتائج التي تم الحصول عليها يمكن استنتاجها بناءً على الفرق بين الدواء الجينسي والدواء المرجعي بالإضافة إلى القيمة المثالية. وكان النتائج كما يلي:

- بالنسبة للدواء المرجعي: $C_{\text{max}}$ = 199.99 ng/ml, $T_{\text{max}}$ = 8.83 hours, AUC$_{0-t}$ = 82.87 ng.hr/ml, AUC$_{0-\infty}$ = 95.23 ng.hr/ml, $C_{\text{max}}$/AUC$_{0-\infty}$ = 0.0219 hr$^{-1}$, $\lambda_z$ = 0.018 hr$^{-1}$, MRT = 38.5 hr, Cl/F = 56.2 hr, Vd/F = 60.9 l/hr, and 3483 liters.

- بالنسبة للدواء الجينسي: $C_{\text{max}}$ = 192.00 ng/ml, $T_{\text{max}}$ = 7.9 hours, AUC$_{0-t}$ = 76.3 ng.hr/ml, AUC$_{0-\infty}$ = 89.31 ng.hr/ml, $C_{\text{max}}$/AUC$_{0-\infty}$ = 0.0225 hr$^{-1}$, $\lambda_z$ = 0.019 hr$^{-1}$, MRT = 36.7 hr, Cl/F = 59.9 hr, Vd/F = 69.5 l/hr, and 3983.4 liters.

و بعد التحليل الإحصائي قبالة الدستور الأمريكي لدراسة التكافؤ الحيوي فقد ثبت أن الدواء الجينسي متكافئ حيويا مع الدواء المرجعي.

الكلمات المفتاحية: الاملودبين, حرکية الدواء, التكافؤ الحيوي, طريقة HPLC/MS/MS.
Introduction

Amlodipine is a long-acting calcium channel blocker. Amlodipine is chemically described as (R.S) 3-ethyl-5-methyl-1-(2- aminoethoxy-methyl) - 4- (2 -chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedi-carboxylate benzenesulphonate. Its empirical formula is: C_{20}H_{26}ClIN_{2}O_{5}.C_{3}H_{4}O_{5}S. The molecular weight is 567.1. Amlodipine is indicated for the treatment of hypertension, chronic stable and vasospastic angina. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of amlodipine is not altered by the presence of food. Amlodipine is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in the urine. Approximately 93% of the circulating drug is bound to plasma proteins. Pharmacokinetics of amlodipine are not significantly influenced by renal impairment and patients with renal failure may therefore receive the usual initial dose. After oral administration of therapeutic doses of amlodipine, absorption produces peak plasma concentrations between 6 and 12 hours. Elimination of amlodipine from plasma is biphasic with a terminal elimination half-life of about 30-50 hours. The therapeutic dose of amlodipine is 2.5-10 mg.

Due to wide use of amlodipine in clinical practice, concomitant use with other drugs, high interindividual variation, in addition to, the pharmacokinetic characteristics which involve slow absorption, lone time to peak and long elimination half-life; many investigations were conducted to study the pharmacokinetics, pharmacody-namics, bioavailability, and bioequivalence of amlodipine after different dosage forms, after food/fluid intake, and in different populations. Determination of amlodipine concentrations in human plasma were achieved by HPLC-MS method due to the low therapeutic doses of amlodipine and consequently very low plasma levels (nanograms/ml). Bioequivalence studies are considered as pivotal part for registration of generic products since these studies are conducted to show that the rate and extent of bioavailability of the generic product is similar to the brand/innovator product. Consequently, the effect(s) and the side effect(s) of the generic product are essentially equivalent to the brand/innovator product, and hence both products are interchangeable in clinical practice.

The purpose of this study was to investigate the pharmacokinetics and relative bioavailability (bioequivalence) of two amlodipine formulations; a test product as tablet containing 5 mg amlodipine, in comparison to Norvasc® 5 mg tablet (Pfizer USA) as the reference product, after administration to 28 healthy male adult subjects applying randomized crossover design.

Materials and Methods

Study products information

A test product as tablet containing 5 mg amlodipine. The reference product was Norvasc® tablet manufactured by Pfizer USA.

Ethical consideration

The study was carried out according to the provisions of the declaration of Helsinki (18) and ICH guidelines for good clinical practice (19). The subjects provided informed consent before the commencement of the study.

Study design

A fasting, single-dose, two-treatment, two-period, two-sequence, randomized crossover design was applied as recommended by FDA guidance for bioavailability and bioequivalence. Twenty eight subjects participated in the study. Equal number of subjects (14 subjects) were randomly assigned to each dosing sequence of the treatments (test and reference formulations). The treatments were separated by two weeks washout interval between period I and period II dosing.

Inclusion criteria

Twenty eight subjects were selected according to the following inclusion criteria: age between 18-48 years; normal Body-Mass-Index (BMI) =18-28; non smokers; no drug or alcohol abuse; no history of contraindication and/or allergy to the drug and any related compounds; normal physical and clinical examinations including vital signs, hepatic, renal, respiratory, cardiac, gastrointestinal and psychiatric; normal clinical laboratory tests including biochemistry, hematology, routine urine analysis, negative for HIV, hepatitis B and C; no consumption of drugs for two weeks prior the study; no blood donation, hospitalization or participation in any study (clinical, pharmacokinetic, bioavailability or bioequivalence) within the last 2 months prior to the present study.

Drug product administration and the conditions of the study

The drug was administered with 240 ml of water after an overnight fasting of 12 hours. No water was permitted 2 hours before and
after dosing. Water was allowed 2 hours after dosing. Standard diets (breakfast, lunch and dinner) were administered and were identical in both periods of the study. Xanthine containing products were not allowed twelve hours before dosing and then twelve hours after dosing. Grapefruit juice or beverages containing grapefruit were not allowed within the past week prior the study and until the completion of the whole study (both periods of the study). The subjects were not allowed to sleep or lie during the first four hours of drug administration, they remained seated upright.

**Blood samples collection**

Seven ml of blood samples were withdrawn via an Indwelling Cannula placed in the forearm antecubital vein at zero time (one hour before dosing), and then at: 1.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 16.0, 24.0, 36.0, 48.0, 72.0, 96.0, 120.0 & 144.0 hours post dosing. The blood samples were directly transferred to heparinized tubes and then immediately centrifuged for 5 minutes at 4000 rpm. The plasma samples were separated by polypropylene disposable tips and transferred to Eppendorf tubes and then immediately stored at -20°C until analysis for determination of amlodipine concentrations in plasma.

**Clinical observations**

Vital signs (blood pressure and pulse) of each subject were measured one hour before dosing and then at 3, 6, 9 and 12 hours post dosing.

**Analytical procedure**

Amlodipine’s concentrations in plasma were determined by a modified HPLC-MS/MS method obtained from previously published HPLC-MS/MS methods (20-23). The validation of the method was evaluated following FDA bioanalytical method validation criteria (24) and GLP guidelines (25). Samples were extracted using liquid-liquid extraction technique. In each run, 1ml of plasma was alkalinized using 6 ml of Hexane: ethyl acetate (1:1) extraction solvent. Diazepam was used as an internal standard. The samples were then shaken for 20 minutes at 250 rpm and later centrifuged for 5 minutes at 4000 rpm. The upper organic layer was aspirated and placed into another tube and then evaporated to complete dryness under nitrogen stream. The residual samples were reconstituted with 50 µl of 1% acetic acid in methanol: water 1:1 to be ready for direct injection into the HPLC. Drug quantitation was done using a Finnigan LCQ DUO ion trap mass spectrometer (Finnigan Thermoquest, USA) equipped with an (ESI) source (Finnegan) and run by: Xcalibur 1.2 software (USA). Calibration standard responses were linear over the range of 0.1-10 ng/ml of amlodipine concentrations in human plasma with a lower limit of quantitation (LLOQ) of 0.1 ng/mL. The plasma samples were analyzed after the completion of the clinical part of the study as recommended in bioequivalence studies. Plasma samples of each subject for both periods were analyzed with their own calibration curve and quality control (QC) samples as one batch in a single run. For each run, six QC samples (dispersed evenly in a low-high and high-low sequence throughout the batch) were analyzed. No determination was done by extrapolation below the LLOQ and above the upper limit of quantitation (ULOQ) of the standard calibration curve as recommended by FDA bioanalytical method validation guidance (24).

**Pharmacokinetic analysis**

Kinectica 2000 (V4.0) software was used for all pharmacokinetic analysis of data. A non-compartmental analysis was applied for all pharmacokinetic calculations as recommended by FDA and EMEA guidance in bioavailability and bioequivalence (26, 27). The pharmacokinetic parameters clearance (Cl), apparent volume of distribution (Vd), elimination rate constant (K), terminal elimination half-life (T 0.5), area under plasma concentration-time curve (AUC), area under moment curve (AUMC), and mean residence time (MRT) were calculated applying standard methods (28).

**Statistical analysis**

Kinectica 2000 software (V4.0) was used for the statistical analysis of data. For the purpose of bioequivalence evaluation (26, 27, 29), analysis of variance (ANOVA), 90% confidence interval and Schuirmann’s two one-sided t-test were applied. ANOVA were carried out to account for the effects of the following sources of variation: treatment, period, sequence and subjects nested in sequence; on the pharmacokinetic parameters; Cmax, Tmax, AUC0-t, AUC0-∞, Cmax/AUC0-∞, λz and T0.5. ANOVA was also executed for the Ln-transformed values of the pharmacokinetic parameters; Cmax, AUC0-t, AUC0-∞, and Cmax/AUC0-∞. The difference between the pharmacokinetic parameters of both products were declared statistically insignificant at 5% significance level (α = 0.05) when P ≥ 0.05. The 90% Confidence Interval (CI) for the ratio of the mean test/mean reference (T/R) for the Ln-transformed values of the pharmacokinetic...
parameters; $C_{max}$, AUC$_{0-t}$, and AUC$_{0-\infty}$ were concluded bioequivalent if the lower CI $\geq 80\%$ and the upper CI $\leq 125\%$, as recommended by FDA guidance in bioavailability and bioequivalence (26, 29). Schuirmann’s two one-sided t-test (30) was also applied for the pharmacokinetic parameters; $C_{max}$, AUC$_{0-t}$, and AUC$_{0-\infty}$ as a check and further support of bioequivalence between both products. Both products were concluded bioequivalent by the Schuirmann’s test if the lower-T (T$_L$) $\geq (T_{0.05} - 26 df)$ and the upper T (T$_U$) $\geq (T_{0.05} - 26 df)$. For the $T_{max}$ values, the parametric point estimate was measured as the difference between the mean values of the test and the reference products. The acceptance limit for the $T_{max}$ was within $\pm 20\%$ of the mean value of the reference product. Nonparametric test was also applied for the $T_{max}$. ANOVA testing was applied for the pharmacokinetic parameters; MRT, CI/F and $V_d/F$ of the test product versus the reference product.

Results and Discussion
Clinical observations
Both test and reference products were well tolerated by all subjects. No incidences of serious side effects or adverse reactions were observed during the study. All the subjects who started the study participated to the end of the study.

Plasma concentrations

The developed LC-MS/MS method presented in this study with LLOQ of 0.1 ng/mL was rapid, sensitive, precise, accurate and specific for quantitation of amlodipine in human plasma. Therefore, the present method can successfully applied to analyze large number of plasma samples for pharmacokinetic, bioavailability and bioequivalence studies of amlodipine in human plasma. No significant differences (P $> 0.05$) in the plasma concentrations were found in all sampling time points between the test and the reference products. One hour before dosing (pre-dose sample), amlodipine was not detected in plasma samples of any subject indicating the absence of carryover effects and insuring a sufficient washout period. The drug was detected in plasma samples of 21 volunteers and 20 volunteers after 1.0 hour post dosing of the test product and Norvasc® tablets, respectively. This indicates rapid appearance of amlodipine in plasma. Figure 1 shows the profiles of the mean amlodipine plasma concentration-time data of the 28 volunteers for each product. This figure indicates that the plasma concentration-time profiles of amlodipine for both products are to a very good extent superimposable.

![Figure 1: Mean plasma concentrations (± SD) of amlodipine after a single dose administration of a test product (tablet containing 5 mg amlodipine) and the reference product (Norvasc 5 mg tablet) to twenty eight healthy male adult subjects.](image-url)
Table (1) Mean (± SD) of pharmacokinetic parameters of amlodipine after a single dose administration of a test formulation (tablet containing 5 mg amlodipine) and the reference formulation (Norvasc® 5 mg tablet) to 28 healthy male adult subjects.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Test Formula</th>
<th>Reference Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>1.99</td>
<td>1.92</td>
</tr>
<tr>
<td>$AUC_{0-t}$ (ng.hr/ml)</td>
<td>82.87</td>
<td>76.30</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng.hr/ml)</td>
<td>95.23</td>
<td>89.31</td>
</tr>
<tr>
<td>$C_{\text{max}}$/AUC$_{0-\infty}$ (hr$^{-1}$)</td>
<td>0.0219</td>
<td>0.0059</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>8.3</td>
<td>7.9</td>
</tr>
<tr>
<td>$T_{\frac{1}{2}}$ (hr$^{-1}$)</td>
<td>0.018</td>
<td>0.019</td>
</tr>
<tr>
<td>$T_{\text{last}}$ (hr)</td>
<td>38.5</td>
<td>36.7</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>56.2</td>
<td>59.9</td>
</tr>
<tr>
<td>$Cl/F$ (l/hr)</td>
<td>60.9</td>
<td>69.5</td>
</tr>
<tr>
<td>$V_d/F$ (l)</td>
<td>3483</td>
<td>3983.4</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ = Maximum concentration of drug in plasma, obtained directly from the concentration versus time curves of individual volunteers.

$T_{\text{max}}$ = The time to attain $C_{\text{max}}$, obtained directly from the concentration versus time curves of individual volunteers.

$AUC_{0-t}$ = Area under the plasma concentration-time curve from time zero to $t_{\text{last}}$, calculated by trapezoidal rule.

$AUC_{0-\infty}$ = Extrapolated area under the plasma concentration-time curve from $t_{\text{last}}$ to infinity, calculated as $C_{\text{last}}/\lambda_Z$.

$AUC_{0-\infty}$ = Total area under the plasma concentration-time curve from time zero to infinity, calculated from the sum of $AUC_{0-t} + AUC_{0-\infty}$.

$\lambda_Z$ = First order terminal elimination rate constant, estimated by linear regression of not less than 3 points of the last points at the terminal phase of the log-concentration versus time curves of individual volunteers.

$T_{\frac{1}{2}Z}$ = First order terminal elimination half-life, equal to $0.693/\lambda_Z$.

$C_{\text{last}}$ = Last measurable concentration which meet or exceed the lower limit of quantitation.

$t_{\text{last}}$ = Time at which $C_{\text{last}}$ occur.

MRT = Mean residence time, calculated as $\text{AUMC}_{0-\infty}/AUC_{0-\infty}$.

$AUMC_{0-\infty}$ = Area under the moment curve from time zero to infinity.

$Cl/F$ = Oral body clearance, calculated as $F \times \text{Dose}/AUC_{0-\infty}$, Dose=5 mg, $F=0.7$.

$V_d/F$ = Oral volume of distribution, calculated as $C_{\text{last}}/\lambda_Z$.

$F$ = Oral bioavailability.

**Statistical evaluation**

ANOVA tests were performed for all the calculated pharmacokinetic parameters presented in Table 1, whereas 90% CI and Schuirmann’s two one-sided t-test (Table 2) were applied only for the pharmacokinetic parameters $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$, since these three parameters are considered as the primary pharmacokinetic parameters for bioequivalence evaluation as recommended by FDA Guidance (26, 29).

ANOVA tests for the $C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $T_{\text{max}}$, $C_{\text{max}}$/AUC$_{0-\infty}$, $\lambda_Z$ and $T_{\frac{1}{2}Z}$ values and for the corresponding Ln-transformed values of $C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-\infty}$, and $C_{\text{max}}$/AUC$_{0-\infty}$ revealed no significant effects (P > 0.05) for the sources of variation: treatment, period and sequence. However, for the subjects nested in sequence, a significant effect (P < 0.05) was found which may be due to the interindividual variation in the above mentioned parameters as shown in Table 1. Moreover, ANOVA tests for MRT, $Cl/F$ and $V_d/F$ showed no significant difference (P > 0.05) between the test and the reference formulas. These findings support the similarity in the pharmacokinetic behaviors of the test and the reference formulas.

The calculated ranges of the 90% CI (Table 2) for the Ln-transformed values of $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were well within the FDA bioequivalence acceptance criteria (26, 29). The ranges of Schuirmann’s two one-sided t-test (Table 2) for the above pharmacokinetic parameters were also well within the bioequivalence acceptance criteria (29, 30). Moreover, power calculations
for C\text{max} and AUC demonstrated that sample size of 28 subjects is adequate to obtain power above 80% for bioequivalence evaluation of amlodipine tablets. Therefore, according to FDA Guidance on bioequivalence, it is concluded from the results of the above statistical tests that the test product and the reference brand product (Norvasc® tablet) are bioequivalent.

### Table (2) 90% Confidence Interval and Schuiramann’s two one-sided T-tests for the pharmacokinetic parameters of the test versus the reference products.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>T/R Geometric Mean Ratio</th>
<th>90% Confidence Interval*</th>
<th>Schuiramann’s Two One-Sided T-Test**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower limit</td>
<td>Upper limit</td>
</tr>
<tr>
<td>C\text{max}</td>
<td>1.03</td>
<td>95.29</td>
<td>109.82</td>
</tr>
<tr>
<td>AUC\text{0-4}</td>
<td>1.07</td>
<td>100.3</td>
<td>115.23</td>
</tr>
<tr>
<td>AUC\text{0-\infty}</td>
<td>1.04</td>
<td>97.76</td>
<td>112.48</td>
</tr>
</tbody>
</table>

* Acceptance criteria = lower limit ≥ 80 and upper limit ≤ 125.0.
** Acceptance criteria = lower limit and upper limit ≥ 1.7081.

### Conclusion
The present study introduced pharmacokinetic characteristics of amlodipine after therapeutic oral dose to healthy male adult subjects. The pharmacokinetics of the test product are statistically similar to the reference brand product (Norvasc® tablet) produced by Pfizer USA. Therefore, according to FDA guidance on bioavailability and bioequivalence, it is concluded that the test product is bioequivalent to Norvasc® tablet. Therefore, both products are interchangeable in therapy with amlodipine, and the test formula can be considered prescribable as Norvasc® tablets produced by Pfizer USA.

### References
1. Physician’s Desk Reference® (PDR), 68th edition, 2014; Norvasc® product information, Pfizer USA.


27. EMEA Guidance on Bioavailability and Bioequivalence, October, 2010.


