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# Bioequivalence of Losartan/Amlodipine Fixed Dose Combination Tablets (Losanet AM) Compared with Concomitant Administration of Single Components of Losartan and Amlodipine Tablets in Healthy Human Volunteers

**Rana Bustami[1], Sewar Khasawneh[1], Wafaa' Absi[1], Hamzeh Feddah[1], Mohamad Mroueh[2], Elie Daccache[3], Jean-Charles Sarraf[3] and Soula Kyriacos[3]***

[1]*Pharmaceutical Research Unit; Clinical Evaluation Centre, Amman, Jordan*
[2]*Pharmaceutical Sciences Department, School of Pharmacy, Lebanese American University, Byblos, Lebanon*
[3]*Research & Development Department, Pharmaline, Jdeidet-El-Metn, Lebanon*

## Abstract

A fixed dose combination of losartan, an angiotensin receptor blocker and amlodipine, a calcium channel blocker, can potentially provide complementary mechanism of action to improve blood pressure control and clinical outcomes. The current study was conducted to compare the pharmacokinetics of a new combination product of losartan potassium and amlodipine besylate with separate co-administration of losartan potassium and amlodipine besylate tablets in 40 healthy human volunteers after a single oral dose in a randomized three-period cross-over study. The study protocol was prepared in accordance to the requirements set in the EMA guidance for conducting bioequivalence studies. Reference (Cozaar 100 mg, Merck Sharp & Dohme Ltd, UK and Norvasc 10 mg, Pfizer, Canada) and test (Losanet AM, Pharmaline, Lebanon) drugs were administered to fasted volunteers and blood samples were collected up to 168 hours and assayed for losartan, carboxylic acid losartan metabolite and amlodipine using a validated LC-MS/MS method. The pharmacokinetic parameters $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$, $T_{max}$, $T_{1/2}$, $MRT_{inf}$, residual area (%) and elimination rate constant were determined from plasma concentration-time profile by non-compartmental analysis method using WinNonlin V5.3. The analysis of variance did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range for bioequivalence (80-125%). The resulting data demonstrated that when administered as fixed dose combination or individual tablets, the pharmacokinetics of losartan and amlodipine were bioequivalent and were well-tolerated.

**Keywords:** Losartan; Amlodipine; Pharmacokinetics; Bioequivalence

## Introduction

Hypertension is a chronic condition that affects about 1 billion people and is the number one risk factor for premature death worldwide [1]. It is associated with an increased risk of heart attack, stroke, heart failure, kidney disease and death [1-3]. Several guidelines for the pharmacological management of hypertension depending on the severity of the disease have been published [4-8]. Different classes of antihypertensive drugs used to treat hypertension are recommended including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), diuretics, beta-blockers, calcium channel blockers (CCBs) and others depending on the cause of hypertension. These drugs are used as monotherapy or in combination. American and international guidelines acknowledge that most patients need combination therapy to achieve blood pressure (BP) goals. Advantages of combinations over monotherapy in the treatment of hypertension include: faster achievement of target BP, greater efficacy, higher response rates, improved outcomes, increased potential for end organ protection, potential for fewer side effects, simple convenient regimen, improved compliance and lower treatment costs [9,10]. Among combination regimens are the ARBs with CCBs which lead to synergistic BP reduction and to complementary clinical benefits [10-13]. Inhibition of the RAAS system is usually required to get the most effective BP lowering with combination therapy; CCBs are vasodilators and can activate the RAAS which limits their effectiveness; reflex activation of the sympathetic nervous system by CCB-induced vasodilatation is diminished by ARBs; ARBs decrease the adverse effects associated with CCBs (e.g. peripheral edema). ARBs and CCBs are therefore recommended for complementary indications. ARBs confer stroke protection, cardiac protection, renal protection, and tolerability similar to placebo, without dose-related symptomatic and metabolic adverse events. CCBs are beneficial in reducing stroke and treating angina and cardiac ischemia.

The combination of an ARB with a CCB as a single pill, fixed dose treatment is emerging as possibly the best therapy for preventing cardiovascular disease. It is important to highlight that some benefits conferred by ARBs may not be class effects but rather molecular effects. Losartan, the first FDA approved ARB, is the most extensively studied ARB and has more approved indications as compared to other ARBs [14]. In addition, losartan is devoid of dry cough and angioedema as compared to its ACE-inhibiting counterparts.

Losartan and its principal active metabolite (EXP3174) block the AT1 receptor which mediates vasoconstriction and aldosterone-secretion, leading to vasodilation and decrease sodium and water retention [15]. According to the Biopharmaceutics classification

***Corresponding author:** Soula Kyriacos, R&D Manager, Pharmaline P.O. Box 90201, Jdeidet-El-Metn, Lebanon, Tel: 9619440901; Fax: 9619448418; E-mail: soulakyriacos@maliagroup.com

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system [16], losartan is a Class 3 drug as it exhibits a high solubility and low permeability [17]. It is a well absorbed orally active agent that undergoes substantial first-pass metabolism by CYP450 enzymes with a systemic bioavailability of approximately 33%. It is converted, in part, to an active 5-carboxylic acid metabolite which is 10 to 40 times more potent than losartan (by weight) and appears to be a reversible, non-competitive inhibitor of the AT1 receptor. While maximum plasma concentrations of losartan and its active metabolite are approximately equal, the AUC of the metabolite is about 4 times as great as that of losartan. Food slows absorption of losartan and decreases its peak plasma concentration but has minimal effect on AUC of losartan or its active metabolite. Following oral administration of a single dose of losartan, about 4% of the dose is excreted unchanged in the urine and about 6% is excreted in urine as active metabolite. Biliary excretion contributes to the elimination of both parent drug and active metabolite. The pharmacokinetics of losartan and EXP3174 are linear with oral losartan doses up to 200 mg and neither one accumulate in plasma upon repeated once-daily dosing [14,18].

Amlodipine is a calcium ion influx inhibitor of the dihydropyridine group (slow channel blocker or calcium ion antagonist). The mechanism of the antihypertensive action of amlodipine is due to a direct relaxant effect on vascular smooth muscle. Amlodipine dilates peripheral arterioles and veins thus, reduces the total peripheral resistance (afterload and preload). Since the heart rate remains stable, this unloading of the heart reduces myocardial energy consumption and oxygen demand. Another mechanism of action of amlodipine involves dilatation of coronary arteries, increasing oxygen supply, both in normal and ischemic regions [19].

According to the Biopharmaceutics Classification System, amlodipine is a Class 1 drug as it exhibits high solubility and high permeability [17]. After oral administration of therapeutic doses, amlodipine is well absorbed. Absolute bioavailability has been estimated to be between 64 and 90% and is not altered by food intake. It is extensively converted to inactive metabolites in the liver (about 90%) with 10% of the parent compound and 60% of the metabolites undergo renal excretion. Pharmacokinetic parameters are not significantly influenced by renal impairment [20,21].

This bioequivalence study was performed using an in vivo method where the concentrations of the active ingredient and metabolite in an accessible biological fluid are measured as a function of time in humans. According to EMA, this is the most recommended method to assure that formulations perform in an equivalent manner and thus demonstrate that they are therapeutic equivalent [22]. This bioequivalence study was performed on the highest strength available. The medicine is available in two strengths: losartan potassium 100 mg/amlodipine (as besylate) 10 mg tablets and losartan potassium 100 mg/amlodipine (as besylate) 5 mg tablets.

Inter-subject pharmacokinetic variability for amlodipine and losartan is about 14.7% and 42.5% respectively. Multiple dose pharmacokinetics are predictable from single-dose data, [18,21].

## Objectives

This study was conducted to assess the bioequivalence of a new fixed dose combination (FDC) including losartan potassium and amlodipine besylate (Losanet AM, Pharmaline, Lebanon) with coadministration of losartan potassium and amlodipine besylate as separate tablets, (respectively Cozaar 100 mg, Merck Sharp & Dohme Ltd, UK and Norvasc 10 mg, Pfizer, Canada). The study protocol was prepared with relevance to the requirements set in the EMA CPMP (CPMP/EWP/QWP/1401/98 Rev 1 [22] guidance for conduction of bioequivalence studies.

## Materials and Methods

### Study products

Investigational product: Losanet AM – Losartan potassium 100 mg and Amlodipine (as besylate) 10 mg tablets

Batch no.: LABE2011 Expiry Date: October/2013

Manufacturer: Pharmaline, Lebanon

Reference product: Cozaar - Losartan potassium 100 mg tablets

Batch no.: NP03390 Expiry Date: October/2013

Manufacturer: MSD, UK

Reference product: Norvasc - Amlodipine as besylate 10 mg tablets

Batch no.: A10469532 Expiry Date: August/2014

Manufacturer: Pfizer, Canada

### Study design

Forty two healthy, Caucasian adult males participated in this comparative study at PRU, Amman, Jordan. Although the study targeted, as per the protocol inclusion criteria, the general population, no female showed up at the site. The sample size calculation was based on 40% intra-subject variability of $C_{max}$ and 80% power to detect 20% difference between the two formulations [18, 21, 23, 24]. The mean age was 28 ± 8 years with a range of 18 to 48 years old, mean body weight was 77 ± 10 kg with a range of 58-102 kg, mean body height was 176 ± 6 cm with a range of 164-192 cm and body mass index was 25.1 ± 2.1 kg/m$^2$ with a range of 21.3-30.0 kg/m$^2$. The volunteers had medical history and physical examination within the range of clinical acceptability, and laboratory results (hematology, blood biochemistry, and urine analysis) within normal ranges. All subjects were instructed to abstain from taking any drug including vitamins and herbal supplements for 14 days prior to first dosing and during the study period. They were informed about the aim and risks of the study by the clinical investigator and then signed a written informed consent statement before entering the study. The study was carried out in accordance with the principles enunciated in the Declaration of Helsinki resolved in Helsinki in 1964 and amended in Seoul, 2008 [25], the ICH harmonized tripartite guideline regarding Good Clinical Practice [26], OECD Principles of Good Laboratory practices [27] and the local requirements of the Jordan Food and Drug Administration in relation to human rights and confidentiality [28]. Before the start of the study, the protocol was approved by the Institutional Review Board (IRB) of PRU, Amman, Jordan.

### Drug administration and sample collection

The study was designed as an open-label, randomized, single dose, two-treatment, three-sequence, three-period, and crossover design performed under fasting conditions. The study flow chart (Figure 1) summarizes the study procedure.

Each period lasted 168 hours. The three periods were separated from each other by a washout period of 21 days. Subjects were admitted to PRU clinical site approximately 12 hours prior to study drug administration, until 24 hours after dosing in each period. The subjects returned to the site to give the 36.00 hour and remaining samples as per schedules time. Following an overnight fasting of at least 10 hours, the

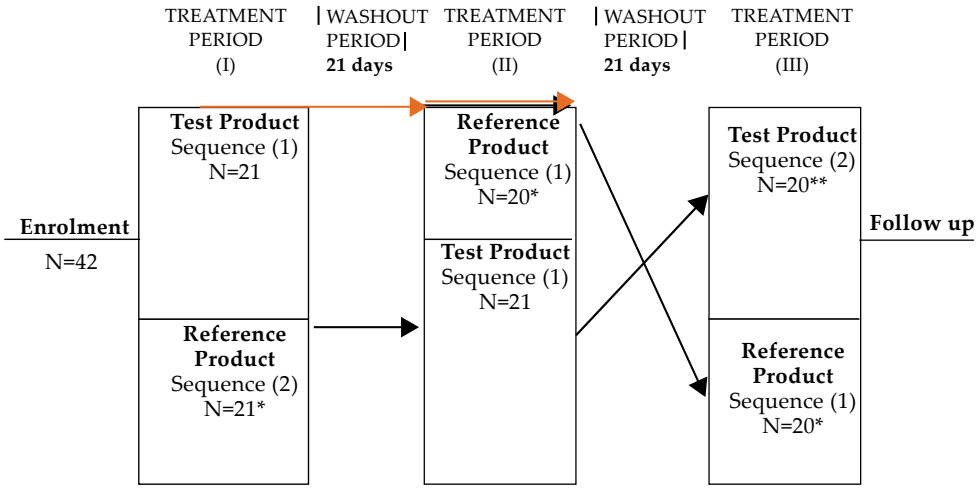


**Figure 1:** Study Flow Chart.

study subjects were given single dose of either formulations (reference or test) of losartan potassium 100 mg and amlodipine as besylate 10 mg with 240 ml of water. No food was allowed until 4 h after dose administration. Lunch and dinner were given to all volunteers according to a time schedule; breakfast was served after the 24 hours sample was collected. Water intake was allowed one hour after the dosing; there was no restriction on water intake 4 hours after drug administration. The volunteers were continuously monitored throughout the confinement period of study. They were not permitted to lie down or sleep for the first six hours after the dose. Blood samples were collected in each study period before (1.00 hour pre-dose) and at 0.25, 0.50, 1.00, 1.33, 1.66, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 6.00, 7.00, 8.00, 9.00, 10.00, 11.00, 12.00, 16.00, 24.00, 36.00, 48.00, 72.00, 144.00 and 168.00 hours after dosing. For each sample, 8 ml of blood for losartan and amlodipine assay were drawn into heparinated tubes through indwelling cannula. Blood samples were centrifuged at 4000 rpm for 5 minutes. The resulting plasma was immediately stored at -70°C until assayed. After a washout period of 21 days the study was repeated in the same manner to complete the crossover design.

## Analysis for parent drugs

**Losartan and its metabolite:** Although bioequivalence acceptance criteria were only based on parent compound pharmacokinetic parameters, assessing losartan metabolite data was done as supportive evidence of comparable therapeutic outcome. Losartan, losartan carboxylic acid (LCA) and the internal standards (Losartan-d4 and Losartan-d4 carboxylic acid (LCA-d4)) were extracted from human plasma samples by protein precipitation using acetonitrile. Fifty microliters of each internal standard working solution (1.682 µg /mL and 1.656 µg /mL respectively) were added to 0.200 ml of plasma sample and vortexed for 30s. Then 1 mL acetonitrile was added to the sample which was subjected to vortex for 30 seconds followed by decantation of the supernatant layer. Ten microliters were injected to the column.

Analysis was performed using a method fully developed and validated at PRU Bio-analytical laboratory. The study plasma samples were analyzed using a validated LC coupled with MS-MS detector. All solvents used were of HPLC grade. Acetonitrile and Methanol were purchased from Honywell (USA). Iso propanol was purchased from Carbon Group (USA). Formic Acid (FA) was purchased from Merck (Germany). Losartan potassium was obtained from Pharmaline whereas the internal standard was from TRC (Canada).

The LC-MS-MS consisted of liquid chromatographic system (Agilent 1200 infinity, USA) , coupled with a triple quadrupole spectrometer (API 4000) from Applied Biosystems, (MDS Sciex, Canada), equipped with ESI source for the ionization (positive ionization mode). Integration was done using the Analyst 1.5.2 software (Applied Biosystems).

Chromatographic separation for Losartan was performed using Phenomenex Luna C18 (3 µm) (50 × 3.00 mm) column from GL sciences, Japan. The mobile phase consisted of acetonitrile, water mixture (60:40) with 0.05% FA and eluted at a rate of 0.500 (mL/min) with splitter (2/3) out for losartan. A flushing solution including acetonitrile, water, 2-propanol and FA mixture (30:40:30:0.05) was used. The auto-sampler temperature was 15°C whereas the column temperature was 30°C. Detection was done by multi reaction monitoring (MRM) mode, using the positive mode. The ion transition (m/z) for losartan was: 423.140/207.100. The ion transition for the internal standard (Losartan-d4) was: 427.199/211.100. The ion transition (m/z) for LCA was: 437.063/235.100. The ion transition for the internal standard (LCA-d4) was: 441.107/239.200. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Analyst software.

Method development and validation were conducted in accordance with international guideline [29]. Under the described conditions, the lower limit of quantitation from 200 µl plasma was 2.929 ng/ml for losartan and 3.469 ng/ml for LCA. Linearity was evaluated by calculating the linear regression (product moment correlation coefficient, r), and by evaluating the back calculated concentrations of the calibration standards. Results of the calibration curve linearity are summarized in Table 1a and Table 1b.

The relationship between concentration and peak area ratio was found to be linear within the range of 2.929-761.605 ng/ml and 3.469 – 901.966 ng/ml for losartan and for LCA respectively. Accuracy and precision were verified using quality control samples at low, medium, high concentration as well as at the LLOQ. Results are reported in Table 1a, and Table 1b.

**a**

| Regression equation | Concentration range (ng/ml) | | Number of points | | Correlation coefficient | | Precision (%) | |
|---|---|---|---|---|---|---|---|---|
| | 2.929-761.605 | | 8 | | 0.9990 | | **6.38%** | |
| **Plasma sample** | **2.929 ng/ml** | | **8.788 ng/ml** | | **380.803 ng/ml** | | **571.204 ng/ml** | |
| | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) |
| Intra-batch (n=6) | 91.40 | 3.92 | 95.38 | 7.55 | 102.57 | 3.18 | 103.08 | 2.35 |
| Inter-batch (n=12) | 95.73 | 6.56 | 96.46 | 6.03 | 102.08 | 3.64 | 105.61 | 5.43 |
| Short-term stability (n=6) | - | - | 97.64 | 2.42 | - | - | 95.24 | 2.27 |
| Freeze and thaw stability (n=6) | - | - | 101.93 | 3.71 | - | - | 103.42 | 2.35 |

**b**

| Regression equation | Concentration range (ng/ml) | | Number of points | | Correlation coefficient | | Precision (%) | |
|---|---|---|---|---|---|---|---|---|
| | 3.469-901.966 | | 8 | | 0.9988 | | **3.44%** | |
| **Plasma sample** | **3.469 ng/ml** | | **10.398 ng/ml** | | **450.583 ng/ml** | | **675.874 ng/ml** | |
| | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) |
| Intra-batch (n=6) | 97.61 | 8.21 | 100.71 | 3.02 | 106.51 | 2.22 | 104.81 | 1.21 |
| Inter-batch (n=12) | 103.57 | 9.32 | 100.13 | 4.35 | 105.80 | 3.36 | 107.69 | 4.88 |
| Short-term stability (n=6) | - | - | 94.94 | 3.72 | - | - | 99.39 | 0.98 |
| Freeze and thaw stability (n=6) | - | - | 93.59 | 5.64 | - | - | 106.71 | 1.97 |

**c**

| Regression equation | Concentration range (ng/ml) | | Number of points | | Correlation coefficient | | Precision (%) | |
|---|---|---|---|---|---|---|---|---|
| | 0.118-9.447 | | 8 | | 0.9967 | | **13.86%** | |
| **Plasma sample** | **0.118 ng/ml** | | **0.354 ng/ml** | | **4.724 ng/ml** | | **7.085 ng/ml** | |
| | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) |
| Intra-batch (n=6) | 94.07 | 12.61 | 97.46 | 8.99 | 110.99 | 2.82 | 104.26 | 2.29 |
| Inter-batch (n=18) | 100.85 | 15.13 | 98.02 | 9.80 | 107.11 | 6.80 | 99.65 | 4.87 |
| Short-term stability (n=6) | - | - | 108.19 | 9.66 | - | - | 95.62 | 1.93 |
| Freeze and thaw stability (n=6) | - | - | 98.02 | 14.12 | - | - | 99.53 | 1.36 |

**Table 1:** Linearity, accuracy, precision and stability data for the analytical method validation for the determination of losartan (1a), LCA (1b) and amlodipine (1c) in plasma

The intra-day accuracy of the method for losartan ranged from 91.40 to 103.08%, while the intra-day precision ranged from 2.35 to 7.55%. The inter-day accuracy for losartan ranged from 95.73 to 105.61% while the inter-day precision ranged from 3.64 to 6.56%. Absolute recovery - percentage ratio of the concentrations in an extracted plasma sample with the reference sample of the same concentration which was dissolved in the same solution as extracted sample - was between 111.70 and 115.87% for losartan and 100.83% for losartan internal standard. For LCA, absolute recovery was between 97.10 and 108.55% and for LCA-d4 135.75%. Accuracy ranged between 100.13% and 107.69%. Short-term temperature stability study demonstrates that losartan and losartan carboxylic acid metabolite are stable in plasma for 24 hours on the bench at room temperature, as shown in Table 1a and in Table 1b. In addition, long term stability studies showed that losartan and losartan carboxylic acid metabolite in the plasma samples were stable when stored frozen for 32 days at both freezer of temperature range between -80°C to -60°C as well as between -25°C to -15°C. Freeze and thaw stability test was determined after four cycles: after storage at temperature range of -80°C to -60°C and -25°C to -15°C for at least 12 hours (or 24 hours for the first cycle), samples were completely thawed unassisted at room temperature. The stability results indicate that losartan and losartan carboxylic acid metabolite are stable under fifth freezing and thawing cycles at both temperature ranges. Table 1a and table 1b report the results after storage at temperature range of -80°C to -60°C.

**Amlodipine:** Amlodipine and the internal standard (Amlodipine-d4 maleic acid salt) were extracted from human plasma samples by liquid-liquid extraction using a mixture (70:30) of diethyl ether (DEE) and dichloromethane (DCM). Fifty microliters of internal standard Amlodipine d-4 working solution (77.662 ng /mL) were added to 0.500 ml of plasma sample and vortexed for 30s. Then 4.00mL (DEE: DCM) (7:3) were added followed by sample shaking for 20 minutes after which samples are centrifuged for 10 minutes at 4000 rpm. Samples were then evaporated under steam of nitrogen at 40˚C and then reconstituted with 150 µL mobile phase. Twenty microliters were injected to the column.

Analysis was performed using a method fully developed and validated at PRU Bio-analytical laboratory. The study plasma samples were analyzed for amlodipine using a validated LC coupled with MS-MS detector (API4000). All solvents used were of HPLC grade; Acetonitrile was purchased from Honywell (USA). Methanol and Iso propanol was purchased from Carbon Group (USA). Ammonium acetate was purchased from Merck (Germany). Diethyl ether was purchased from JHD (India). Dichloromethane was purchased from Pharmco (USA). Amlodipine was obtained from Pharmaline whereas the internal standard Amlodipine-d4 Maleic Acid Salt was from TRC (Canada).

The LC-MS-MS was consisted of liquid chromatographic system (Agilent 1200 infinity, USA) , coupled with a triple quadrupole spectrometer (API 4000) from Applied Biosystems, (MDS Sciex, Canada), equipped with ESI source for the ionization (positive ionization mode). Integration was done using the Analyst 1.5.2 software (Applied Biosystems).

Chromatographic separation was performed using Agilent XDB C18 (5 µm) (150 × 4.6 mm) column from GL sciences, Japan.

The mobile phase consisted of acetonitrile, methanol and 0.008 M ammonium acetate mixture (50:30:20) and eluted at a rate of 1.00(mL/min). Detection was done by multi reaction monitoring (MRM) mode, using the positive mode. The ion transition (m/z) for amlodipine was: 409.100/238.100. The ion transition for the internal standard (amlodipine-d4) was: 413.158/238.000. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Analyst software.

Method development and validation were conducted in accordance with international guideline [29]. Under the described conditions, the lower limit of quantitation from 500 µl plasma was 0.118 ng/ml for amlodipine. Linearity was evaluated by calculating the linear regression (product moment correlation coefficient, r), and by evaluating the back calculated concentrations of the calibration standards. Results of the calibration curve linearity are summarized in Table 1c.

The relationship between concentration and peak area ratio was found to be linear within the range of 0.118-9.447 ng/ml. Accuracy and precision were verified using quality control samples at low, medium, high concentration as well as at the LLOQ. Results are reported in Table 1c.

The intra-day accuracy of the method for amlodipine ranged from 94.07 to 110.99%, while the intra-day precision ranged from 2.29 to 12.61%. The inter-day accuracy for amlodipine ranged from 98.02 to 107.11% while the inter-day precision ranged from 4.87 to 15.13 %. Absolute recovery - percentage ratio of the concentrations in an extracted plasma sample with the reference sample of the same concentration which was dissolved in the same solution as extracted sample was between 93.24 and 103.40% for amlodipine and 106.92% for the internal standard. Short-term temperature stability study demonstrates that amlodipine in plasma is stable for 24 hours on the bench at room temperature, as shown in Table 1c. In addition, long-term stability studies showed that amlodipine in plasma samples was stable when stored frozen for 131 days at both freezer of temperature range between -80°C to -60°C as well as between -25°C to -15°C. Freeze and thaw stability test was determined after five cycles: after storage at temperature range of -80°C to -60°C and -25°C to -15°C for at least 12 hours (or 24 hours for the first cycle), samples were completely thawed unassisted at room temperature. The stability results indicate that amlodipine is stable under fifth freezing and thawing cycles at both temperature ranges. Table 1c reports the results after storage at temperature range of -80°C to -60°C.

### Pharmacokinetic analysis

Pharmacokinetic analysis was performed using the WinNonlin® Computer Program Version 5.3 (Pharsight, USA). The elimination rate constant (λz) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. Elimination half-life ($T_{1/2}$) was calculated as 0.693/ λz. Area under the plasma concentration versus time curve ($AUC_{0-t}$), from time (0) to the last measurable concentration (t), was calculated by the linear trapezoidal method. The area under the plasma concentration versus time curve from time (0) to infinity ($AUC_{0-\infty}$) is calculated as the sum of the $AUC_{0-t}$ plus the ratio of the last measurable plasma concentration to the elimination rate constant. The residual area was determined as the ratio of the difference in the two areas under the plasma concentration versus time curve over $AUC_{0-\infty}$. The mean residual time from zero to infinity is calculated from the ratio of the area under the first moment curve to area under the plasma concentration versus time curve.

### Statistical analysis

The pharmacokinetic parameters $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ were considered as primary variables. Two-way analysis of variance for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. Difference between two related parameters was considered to be statistically significant for p-value equal to or less than 0.05. Parametric 90% confidence intervals based on the ANOVA of the mean test / reference (T/R) ratios of AUCs and $C_{max}$ were computed, [30]. The data was transformed prior to analysis using a logarithmic transformation. $T_{max}$ adopted a non-parametric and was applied to untransformed data.

## Results and Discussion

Out of the forty two volunteers, forty subjects completed the study. One subject was excluded by clinical investigator due to fever, cough, sore throat and headache before dosing of period III. Before admission in period II, another subject was withdrawn from the study due to positive drug abuse (benzodiazepines). Samples for all the subjects who completed the clinical part of the study were analyzed and included in the pharmacokinetic analysis and bioequivalence assessment. Losartan and amlodipine were well tolerated; unexpected incidents that could have influenced the outcome of the study did not occur. All volunteers were discharged in good health.

Both formulations were readily absorbed from the gastrointestinal tract: first quantifiable plasma concentrations of amlodipine and losartan were observed at 0.25 hour; and at 0.50 hour for LCA. Amlodipine and losartan were measurable at least at 1.0 hour in all volunteers. The mean-concentration-time profiles for losartan, LCA and amlodipine for the two formulations are shown in Figures 2-4. Means of peak concentration of 462.247 and 431.910 ng/ml for losartan were attained at means of 1.11 and 1.31 hour after drug administration and then declined rapidly. No losartan was detectable at 48 hours for most volunteers. Losartan concentrations were quantifiable to 144.00 hours in 1 subject after the test product administration and in 1 subject at 72.00 hours after the reference product administration.

Means of peak concentration of 602.092 and 632.874 ng/ml for LCA metabolite were attained at means of 3.27 and 3.34 hour after drug administration and then declined gradually. No carboxylic acid losartan metabolite was detectable after 144 hours.

Means of peak concentration of 6.167 and 5.914 ng/ml for amlodipine were attained at means of 6.14 and 6.52 hour after drug administration and then declined gradually. Amlodipine was still detectable at 168 hours.

Tables 2a, 2b and 2c report the pharmacokinetic parameters of losartan, LCA metabolite and amlodipine for the two brands which highlights the closeness of the results. Mean and standard deviation of the three pharmacokinetic parameters of the two formulations did not differ significantly.

More variability in $C_{max}$ is expected as measurement relies on one single point and not on several points as with the area under the curve. For losartan, the relative bioavailability of Losanet AM on the basis of Cozaar is 883.184 ± 556.879% for $AUC_{0-t}$, 865.997 ± 419.871% for $AUC_{0-\infty}$, and 431.910 ± 259.093% for $C_{max}$. For LCA, the relative bioavailability of Losanet AM on the basis of Cozaar is 4032.223 ± 1991.174% for $AUC_{0-t}$, 4100.329 ± 2014.905% for $AUC_{0-\infty}$, and 632.874 ± 353.512% for $C_{max}$. Data on LCA metabolite was used as supportive data only. For amlodipine, the relative bioavailability of Losanet AM on the basis of

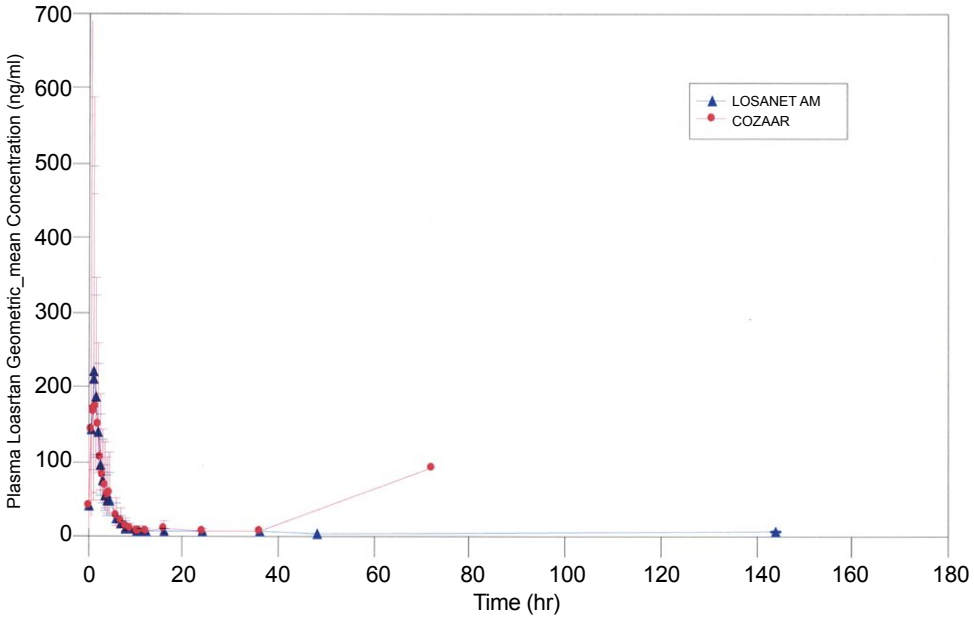


**Figure 2:** Geometric mean (SD) plasma concentration-time profiles for losartan following administration of FDC tablets (Losanet AM) and coadministration of losartan (Cozaar) and amlodipine individual tablets to 40 healthy human volunteers.

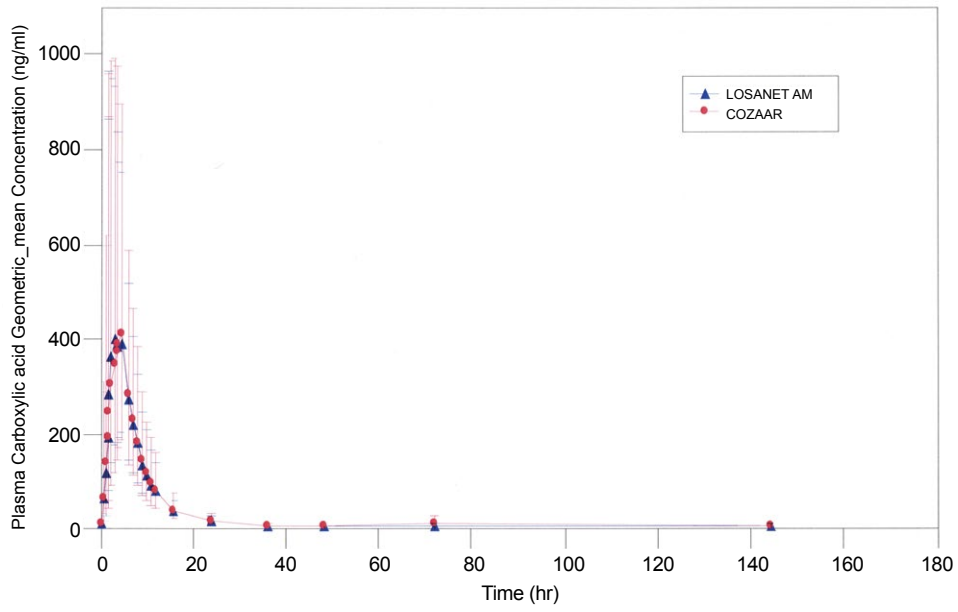


**Figure 3:** Geometric mean (SD) plasma concentration-time profiles for LCA following administration of FDC tablets (Losanet AM) and coadministration of losartan (Cozaar) and amlodipine individual tablets to 40 healthy human volunteers.

Norvasc is 311.535 ± 106.136% for $AUC_{0-t}$, 353.515 ± 123.080% for $AUC_{0-\infty}$, and 5.914 ± 1.329% for $C_{max}$. For bioequivalence assessment, the test & reference products are considered bioequivalent if the 90% confidence interval of the geometric mean of the log-transformed data of the test/reference ratio percentage for amlodipine and for losartan fall within 80.00-125.00 % for each of the primary end points. As reported in Table 3a, 3b and 3c, confidence intervals for amlodipine primary pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were 99.31-107.04%, 100.83-110.94% and 101.25-109.21% respectively. For losartan, $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ values were 87.77-114.60%, 88.61-101.04% and 91.21-102.55% respectively. For LCA metabolite, Cmax, AUC0-t and AUC0-∞ values were 88.62-100.30%, 92.26-99.01% and 93.30-99.89% respectively. All values were within the accepted 80-125% range. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, reveals no statistically significant difference between the two formulations, with p-value greater than 0.05. ANOVA analysis of the drug demonstrates that the sequence, product and period effect for all bioequivalence metrics did not influence the outcome of the study.

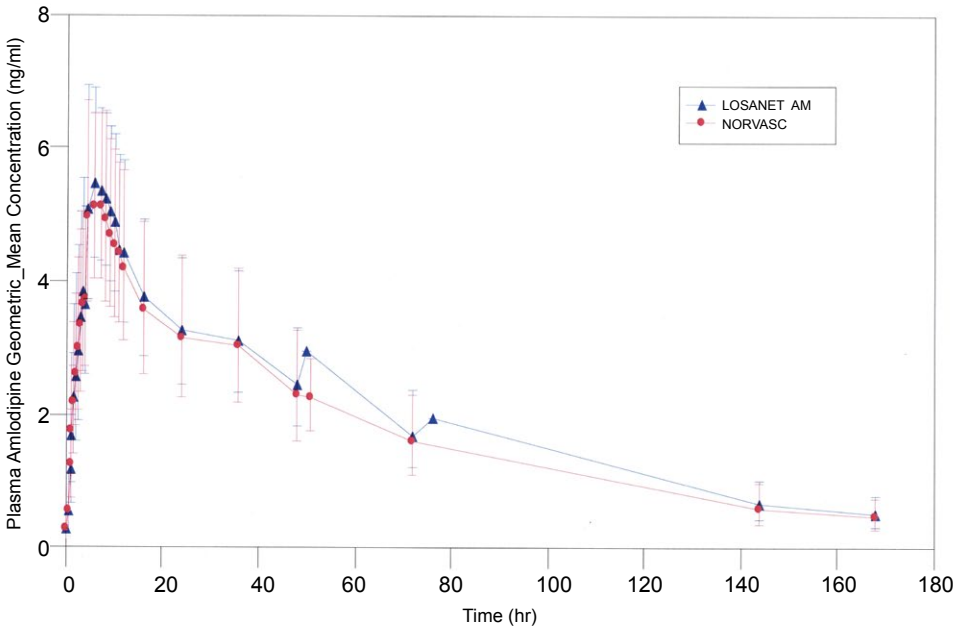


**Figure 4:** Geometric mean (SD) plasma concentration-time profiles for amlodipine following administration of FDC tablets (Losanet AM) and coadministration of losartan and amlodipine (Norvasc) individual tablets to 40 healthy human volunteers.

**a**

| Pharmacokinetic Parameter | Losanet AM 100/10 mg tablets (Test) | Cozaar 100 mg tablets (Reference) |
|---|---|---|
| Cmax (ng /ml) | 462.247 ± 332.039 | 431.910 ± 259.093 |
| AUC 0-t (ng*hr/ml) | 824.602 ± 404.889 | 883.184 ± 556.879 |
| AUC 0-∞ (ng*hr/ml) | 854.606 ± 450.241 | 865.997 ± 419.871 |
| Tmax (hr) | 1.11 ± 0.81 | 1.31 ± 0.86 |
| T1/2 (hr) | 3.82 ± 10.16 | 2.47 ± 1.19 |
| Kelimination (hr-1) | 0.3118 ± 0.0949 | 0.3199 ± 0.0893 |
| MRTinf (hr) | 5.28 ± 16.04 | 3.38 ± 1.42 |
| Residual area (%) | 2.599 ± 4.582 | 2.212 ± 2.756 |

**b**

| Pharmacokinetic Parameter | Losanet AM 100/10 mg tablets (Test) | Cozaar 100 mg tablets (Reference) |
|---|---|---|
| Cmax (ng /ml) | 602.092 ± 327.098 | 632.874 ± 353.512 |
| AUC 0-t (ng*hr/ml) | 3911.388 ± 1780.929 | 4032.223 ± 1991.174 |
| AUC 0-∞ (ng*hr/ml) | 4002.867 ± 1820.782 | 4100.329 ± 2014.905 |
| Tmax (hr) | 3.27 ± 1.27 | 3.34 ± 1.26 |
| T1/2 (hr) | 8.72 ± 7.76 | 7.02 ± 3.19 |
| Kelimination (hr-1) | 0.1046 ± 0.0364 | 0.1096 ± 0.0295 |
| MRTinf (hr) | 10.64 ± 7.99 | 9.09 ± 2.43 |
| Residual area (%) | 2.958 ± 4.876 | 2.486 ± 3.741 |

**c**

| Pharmacokinetic Parameter | Losanet AM 100/10 mg tablets (Test) | Norvasc 10 mg tablets (Reference) |
|---|---|---|
| Cmax (ng /ml) | 6.167 ±1.382 | 5.914 ±1.329 |
| AUC 0-t (ng*hr/ml) | 327.518 ±96.181 | 311.535 ±106.136 |
| AUC 0-∞ (ng*hr/ml) | 370.380 ±114.175 | 353.515 ±123.080 |
| Tmax (hr) | 6.14 ±1.86 | 6.52 ±1.99 |
| T1/2 (hr) | 51.29 ±10.77 | 51.24 ±10.66 |
| Kelimination (hr-1) | 0.0141 ±0.0028 | 0.0141 ±0.0031 |
| MRTinf (hr) | 72.69 ±14.67 | 72.18 ±14.76 |
| Residual area (%) | 11.129 ± 4.745 | 11.806 ±7.579 |

**Table 2:** Pharmacokinetic parameters of losartan (2a), LCA (2b) and amlodipine (2c) for two brands (mean ± standard deviation, n=40)

**a**

| Assessment Parameter | $C_{max}$ | $AUC_{0-t}$ | $AUC_{0-\infty}$ |
|---|---|---|---|
| Point Estimate (%) | 100.29 | 94.62 | 96.71 |
| Lower limit (%) | 87.77 | 88.61 | 91.21 |
| Upper limit (%) | 114.60 | 101.04 | 102.55 |
| Power | 73.56 | 99.98 | 99.50 |

**b**

| Assessment Parameter | $C_{max}$ | $AUC_{0-t}$ | $AUC_{0-\infty}$ |
|---|---|---|---|
| Point Estimate (%) | 94.28 | 95.57 | 95.64 |
| Lower limit (%) | 88.62 | 92.26 | 93.30 |
| Upper limit (%) | 100.30 | 99.01 | 99.89 |
| Power | 99.68 | 100.00 | 100.00 |

**c**

| Assessment Parameter | $C_{max}$ | $AUC_{0-t}$ | $AUC_{0-\infty}$ |
|---|---|---|---|
| Point Estimate (%) | 103.10 | 105.76 | 105.16 |
| Lower limit (%) | 99.31 | 100.83 | 101.25 |
| Upper limit (%) | 107.04 | 110.94 | 109.21 |
| Power | 100.00 | 100.00 | 99.99 |

**Table 3:** Statistical Analysis: 90% confidence intervals of log transformed data of losartan (3a), LCA (3b) and amlodipine (3c), n=40.

The bioequivalence of Losanet AM 100/10 tablets vs coadministration of Cozaar® and Norvasc tablets following a single dose administration of 100 mg losartan and 10 mg amlodipine to healthy adults under fast conditions was demonstrated.

The administration of losartan with amlodipine is common in medical practice. Such combination proved to be effective and safe [31-35] especially that amlodipine-induced edema with associated activation of the RAAS is attenuated by losartan. The LOTHAR study [31] evaluated medium and long term (one year) efficacy, tolerability and metabolic effects of the FDC of amlodipine and losartan compared to amlodipine or losartan alone. The clinical trial was performed with 198 patients in stage 1 and 2 essential hypertension. The fixed

combination was associated with a high antihypertensive efficacy that is sustained in the long term and a low frequency of adverse events. Long-term incidence of leg edema was approximately four-fold lower than that observed with amlodipine alone. The fixed combination did not change glucose and lipid metabolism in the medium or in the long term. Another study [32] compared the blood-pressure lowering efficacy of amlodipine and losartan combination with amlodipine monotherapy after 6 weeks of treatment. The result demonstrates that the amlodipine /losartan combination provides an effective and generally well-tolerated first line therapy for reducing blood pressure in stage 2 hypertensive patients. In addition, a clinical trial [33] was conducted with 320 patients with essential hypertension dosed with either the fixed dose combination or with mono-agent (amlodipine or losartan) for 8 weeks. The study demonstrates that depending on the dose, the FDC resulted in similar BP lowering or significantly greater BP lowering compared with amlodipine or losartan monotherapy, and was determined to be safe and tolerable in this patient population. Evaluation of the efficacy and safety profiles of the FDC was also demonstrated in patients with essential hypertension inadequately controlled on losartan100 mg [34]. Switching to the FDC therapy was associated with significantly greater reductions in BP and superior achievement of BP goals compared with a maintenance dose of losartan 100 mg in this population. Both treatments were well tolerated with comparable safety profiles.

The clinical data on the fixed dose combination of losartan and amlodipine demonstrates that it is associated with a faster achievement of BP goal and greater reductions in BP as well as higher BP response and control rates compared with monotherapy, reduced adverse events and improved patience compliance. The combination is a rational and convenient option in patients who are likely to need multiple drugs to achieve target blood pressure (stage II HTN, diabetes, chronic kidney disease, or cardiovascular disease). Due to its metabolic neutrality, the losartan/amlodipine combination is also a good option for hypertensive patients with diabetes or metabolic syndrome, in addition to other cardiovascular risk factors.

## Conclusion

The bioequivalence of Losanet AM 100/10 losartan and amlodipine combination tablets (Test Product/Pharmaline s.a.l, Lebanon) and Cozaar® 100 mg losartan tablets (Reference Product/ MSD, UK) coadministered with Norvasc 10 mg amlodipine tablets (Reference Product/ Pfizer, Canada) following the administration of a single dose of 100 mg losartan and 10 mg amlodipine to healthy adults under fast conditions was demonstrated. Single dose of the FDC tablet or the individual monotherapies were generally well tolerated. Both the FDC tablet and the coadministration of individual tablets can be considered therapeutic equivalent, and thus they can be substituted for each other without any adjustment in dose or other additional therapeutic monitoring. The study provides support for administration of the FDC tablet as an alternative option to co-administration of the individual tablets with the added benefits of enhanced patient compliance and convenience.

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Authors of the manuscript do not have conflict of interest to declare. The bioequivalence study was conducted by an independent contract research organization.

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